

Revivifying Europe's research

The next European summit at Fontainebleau could be another dog-fight, spend time making speeches about European federation or — more prudently — find a middle course.

EUROPE may not be made in the few weeks ahead, but there is a danger that it could be broken. And certainly the month that lies ahead will be crucial for the whole enterprise. First come the European elections, only the second occasion on which representatives to the European Parliament have been chosen democratically and not simply nominated by their cronies. Then follows the next meeting of European political leaders at Fontainebleau, formally an occasion when the rest of Europe might thank President François Mitterrand of France for having been an energetic president of the Community (which would be true) but in practice more likely to be dominated by two quite different themes — the British Government's demand that it should be given a substantial rebate (say, £1,200 million) on the contributions it paid last year into the common budget, and the more general proposal that Europe, having made a bad job of creating a common market, should now go on to build a federal state. Between the ridiculous and the sublime, the serious opportunities at Fontainebleau are likely to be forgotten.

The survey of the condition of science in Belgium and the Netherlands which appears elsewhere in this issue points more accurately to some of the tasks that need to be attempted on a European scale (see p.491). Two of Europe's smallest states, in recent memory among the most prosperous, find themselves unexpectedly having to struggle to keep up with the times. As elsewhere, public budgets are being cut so as to escape inflation, the problems of catching up with the new technology are daunting and people worry that established institutions and ways of doing civil business may be damaged by too rapid a transition from the past to an uncertain future. In one way or another, the whole of Europe is similarly affected.

The calculation in the early 1950s was that if the common market that is the core of the Treaty of Rome could be made to function, the result would be an industrial propulation as large as any other in the world, prosperous and industrially creative. In the event, the common market has functioned, but only fitfully. In some respects, the arrangements have been an expensive joke (the butter mountain, the wine lake, and all that). In others (as at a host of national customs posts, which innocents may think should long since have disappeared), chauvinism has triumphed over legality. Public purchasing is still largely determined by public authorities, usually governments, in what seems to be the national interest. The result is that Europe is far from being the productive place the founding fathers foresaw. Those meeting at Fontainebleau should spend at least some time reminding themselves that their dream has not come true.

Chauvinism

Where they go from there depends on their estimate of what has gone wrong. To the extent that chauvinism is still an impediment to the efficient division of labour, it must be supposed that not all of the members of the common market have been serious all of the time. Nor are they now. The British Government is not the only political party whose representatives will be fighting next week's election on a platform which promises that national interests, general or particular, will be fiercely defended at Brussels. Even if these promises are not always kept in full, it is disappointing that they should be though necessary at all. The threatened fight at

Fontainebleau over the British rebate could yet become the signal for the disintegration of the whole experiment.

The remedy, now as at the past two European summits (at Athens and Versailles) is that the participants should be patient, reckoning that their goal is a moving target, and that they should find other things to talk about than the issues on which they are most sharply divided. Here is a shopping list:

- Europe is alarmed at its technical inadequacies, for which reason the European Commission has been putting together programmes for collaborative research and development which must be transnational to attract support. But the Commission has put the cart before the horse. If the division of labour were economical (as one day it may be), industrial companies would shoulder responsibility for applied research, but Europe in general would have an important role to play in the support of more fundamental work, just as national governments now have. So why not begin in this neck of the woods, but slowly, recognizing that it will take time to weld Europe's academic and research community into a single entity, preserving its diversity the while?

- Europe is concerned that an efficient division of labour would leave some member states more prosperous than others in ways that would not automatically be offset by the migration of labour (formally made free under the existing rules, but inhibited by language and inertia). This fear is the seat of many of the restraints on the working of the common market now widely practised. But why not then follow the simple course, followed nationally in most of Europe, of arranging that the socially disadvantaged are somehow compensated from the well-being of others? The European social fund now administered from Brussels is too small to be significant and too discretionary to seem fair.

- Europe is by fits and starts scared stiff that it will once again become a battlefield, a danger made all the sharper by its knowledge that it is neither prosperous nor determined enough to defend itself. There can be no quick solution. The lack of resources is a more pressing restraint than the lack of will, while the problems of approaching the notion of a common defence would reawaken the arguments that made the 1950s wretched. But if the European Communities are to survive and to fulfil their promise, a start has to be made somewhere. Encouraging Europeans to join each others' armed services would be an interesting point, not much canvassed hitherto.

None of this quite lives up to President Mitterrand's eloquent espousal, in Strasbourg the other day, of the European Parliament's plan for a federal Europe, but that is inevitable. The mood has changed a great deal since the day in 1940 when the then British Prime Minister, Winston Churchill, flew to France to propose a political union of the two states. (The offer, hastily conceived, was turned down.) Curiously, life is altogether more serious now that it does not seem that it is about to be snuffed out. But as in any polite society, it is important that President Mitterrand's visitors at Fontainebleau should not make fun of him for his enthusiasm or, worse, suppose that he was not serious (which in some sense may nevertheless be true). The British among Europeans are notoriously given to the practice of a maddening pragmatism, and are at risk of forgetting that if



President Mitterrand were only half serious, he may nevertheless have hit on a way that will appeal to others of circumventing present impediments in Europe. The best hope at Fontainebleau is that both the British and their detractors will find some way of bridging the gap between short and long-term interests. The best field in which to look for a bridging formula is that for wringing from Europe's academic and research institutions the promise of which they are richly capable. □

One treaty to sign

One or other of the nuclear powers should urge that the threshold test-ban treaty be ratified.

ON the face of things, there seems no prospect of an early improvement in relations between the Soviet Union and the West. Even the proposal that the president of the US National Academy of Sciences, Dr Frank Press, should go to Moscow to try out some unspecified plans for further collaboration has been put off by accumulating doubts about the welfare of the Sakharovs (see p.485). This week's economic summit in London, which has brought together many of those who might have been able to influence events, was constructed around a different agenda (and a pretty empty one at that). Next will come the Northern Hemisphere's vacations and then the presidential election campaign in the United States, into which President Reagan will plunge without the once-hoped-for deal with the Soviet Union on some form of arms control. On present form, it will be a full year before anybody who matters will be in a frame of mind to take constructive steps.

Here, then, is a simple recipe for action now, not sometime in the future: dust off the Threshold Test-Ban Treaty and see that it is ratified by the United States and the Soviet Union. The treaty has been signed for the past decade, since when both the major nuclear powers have publicly insisted that they have kept to the letter of the treaty, which forbids the testing of weapons yielding more than the equivalent of 150,000 tons of conventional explosive. (Because of the earlier partial test-ban treaty, such tests must of course be underground.) While there have been complaints in the past few years, especially from the United States, that the treaty has been violated, the seismic evidence on this point is inconclusive or, more accurately, is consistent with the view that both the major powers have kept their unratified bargain with each other and the rest of us.

So why bother to ratify the treaty, in the process running the risk of a refusal by the US Congress? There are several practical reasons, not the least of which is that the full text of the treaty includes provisions for the exchange of seismic data about the neighbourhood of known testing sites that make the remote monitoring of other people's nuclear explosions less ambiguous, perhaps much more precise. It seems strange that neither of the major powers has sought to reap these benefits — or to shame the other by provoking a public declaration of unwillingness to ratify an otherwise agreed document. That, however, is only one of a long list of reasons why reasonable powers should legally endorse a reasonable document.

The threshold treaty may be a pale shadow of, say, an agreement on European missiles or a further instalment of the strategic arms agreements, but it has been negotiated and the others have not. And while it may not obviously be the "substantial" step towards strategic arms control required of the nuclear powers by the non-proliferation treaty, it is much more than the nothing that, on present form, the nuclear powers will have to report to next year's review conference of that treaty. Finally, the threshold treaty offers obvious scope for extension — reductions of the threshold, remote but on-site inspection — that could help to provide later and more ambitious agreements. It should not have escaped the US Administration's notice that a ratified agreement would also give the president something to boast about in November. The trouble is that there is very little time to act, with the party conventions in the United States only weeks away. That is another argument for moving quickly. □

Shoestring research

The problems of British research are epitomized by this week's closure of a laboratory.

WHILE nobody is conspicuously to blame that one of the best-known British laboratories in the neurosciences is to be closed (see p.486), the affair should give pause to those responsible for the management of research in countries such as Britain, but in Britain in particular. And while the Medical Research Council (MRC) is on this occasion lucklessly responsible, there is every reason to expect the laboratories under the wings of other organizations will similarly find themselves in trouble when the time comes to replace a director who has decided (for good personal reasons) to quit. What has happened is a depressing symptom of what is wrong with the condition of British science.

The MRC Neurochemical Pharmacology Unit has its origins in the council's general policy, going back to before the Second World War, that research can be powerfully stimulated in important fields by providing a scientist of distinction with a good laboratory and a team of close colleagues with whom he can expect to work for decades to come. Of necessity, these units are small and also impermanent. If the good scientist should die, retire or simply quit, the case for the perpetuation of the unit may disappear at the same time. Ironically, the Cambridge unit partly owes its existence to the disappointment caused twenty years ago by the departure for the United States of the then newly appointed head of a similar initiative at the University of Birmingham. On this occasion, the council seems sensibly to have decided that the work of its unit at Cambridge was important enough to justify the search for a successor to the departed director. The outcome, by all accounts, has been disappointing. Part of the trouble seems to have been the search committee's confusion about its objectives. Should it seek a more or less classical pharmacologist? An up-to-date neuroanatomist? Or a molecular biologist? The difficulties seem to have been aggravated by some candidates' agony in making up their minds and by other people's choosiness about candidates with whom they would or would not work. In the event, the mere passage of time seems to have put paid to the hope that the unit could be kept alive.

Recriminations will now abound, but are inappropriate. To spend more than two years looking for a suitable person and then to fail to find one may betoken indecisiveness but is more vividly a proof that Britain, even Cambridge, is no longer a kind of mecca for professional people from elsewhere. Those who think otherwise might reflect on the recent fall in the value of the pound sterling (at or below \$1.40), but there are more serious difficulties. A unit of a dozen or so people may be able to make an important mark in some chosen field if it has been welded together by some central instigator, but is otherwise probably too small to be able to hold its own against the competition. An attempt to create in Britain a unit on a scale that would be capable of holding its own in the exciting but competitive game of telling which genes do what in neurones would be doomed to failure now that speed in working out the nucleotide sequence of a gene has apparently become crucial. The particular lesson for MRC is a further narrowing of the scope for setting up research units on the now-familiar pattern. And just as the Science and Engineering Research Council is being forced by circumstances to reconsider its role in the support of high-energy physics, so MRC may soon find that some fields of once-cheap research are beyond its means.

The other lesson, for British research managers of all kinds, is the importance of flexibility. Historically, MRC's units have been successful because it has been possible to found them quickly in emerging fields of interest, and without uprooting people in the process. There will no doubt continue to be scope for entrepreneurship of that kind. But if competitiveness is important, there can be no preordained pattern or scale for the operation of such units. Those which succeed must be encouraged to grow, even in directions not foreseen. Others, less successful than the Cambridge laboratory, must be allowed to lapse. Doing nothing is the worst policy. □

US genetic regulations

Bacterial field trial to go ahead

Washington

TWO weeks after a federal judge halted Dr Steven Lindow's planned field trial of recombinant ice-nucleating bacteria (see *Nature* 24 May, p.296), the Recombinant DNA Advisory Committee (RAC) of the US National Institutes of Health (NIH) approved a virtually identical proposal from Advanced Genetic Sciences Inc. (AGS), at its meeting on 1 June.

The Lindow experiment was blocked by Judge John Sirica in response to a motion for a preliminary injunction filed by anti-genetic-engineering activist Jeremy Rifkin. The judge's ruling also barred RAC from approving any other experiments involving release of recombinant organisms to the environment until a full hearing can be held on Rifkin's claim that RAC must prepare an environmental impact statement before proceeding in this area. According to NIH officials, preparation of such a statement would require at least a year. (On 25 May, the Court of Appeals in Washington upheld Sirica's preliminary injunction against the Lindow experiment.)

Sirica's decision specifically exempted commercial proposals, however, which are not in any case legally bound by RAC decisions, although companies have been submitting proposals to RAC for review in keeping with the standard practice of voluntary compliance with the RAC guidelines.

At last Friday's meeting, Rifkin asked the committee not to establish a "double standard" that would give private companies an unfair advantage over federally-supported research, which is legally bound by RAC decisions. Robert Mitchell, chairman of the committee, responded that he had ruled that RAC would continue to consider commercial proposals, noting that they were specifically exempted from the court's decisions, which in any case applied only to NIH administration, not the RAC itself, which is legally an advisory body to NIH.

RAC took noticeably greater care this time to spell out its reasons for believing that the ice-nucleating experiments do not pose a risk to the environment. Dr Anne Vidaver, a plant pathologist from the University of Nebraska and a consultant on both the Lindow and the AGS proposals, noted that the modified bacteria to be used in the trials — so-called INA⁻, for ice-nucleating activity minus — already exists in nature along with the INA⁺. The INA⁻ are lacking a gene responsible for the ice-nucleating protein, a protein believed to mediate frost damage to plants at temperatures a few degrees below freezing. Vidaver said that in natural populations of

the bacteria (a variety of *Pseudomonas syringae*) as much as 80 per cent may be INA⁻. She said that apparently the only reason for using recombinant DNA techniques for producing the INA⁻, rather than simply culturing naturally-occurring ones, is that natural organisms are not patentable.

Vidaver recommended several modifications in the AGS proposal: an increase in the frequency of monitoring of the field plot, the withholding of approval of continuing experiments involving other, unspecified varieties of INA⁻ *P. syringae* and a limit of the tests initially to one growing season, rather than the three requested. If preliminary data show no problems, she said, approval for additional tests should be given at that time.

Telecommunications

Luxembourg goes for cable

TINY Luxembourg has thrown a spanner into the comfortable world of European telecommunications. In particular, Luxembourg has deeply offended the government of France by announcing that it plans to license a commercial consortium to launch communications satellites capable of delivering television broadcasts to cable networks throughout Europe.

Among European governments, France is the most deeply offended because France has been relying on Radio-Tele-Luxembourg, the commercial radio and television company based in Luxembourg, to pay towards the cost of the direct broadcasting satellites which France plans to launch in the next few years. Although Luxembourg, like other members of the International Telecommunications Union, has been allotted space in geosynchronous orbit for satellites capable of broadcasting signals powerful enough to reach individual receivers, the Grand Duchy appears for the present not to plan to take up this option.

Instead, the government of Luxembourg is planning to license the Société Luxembourgeoise des Satellites (SLS) to launch less powerful telecommunications satellites. The company, whose chairman is Mr Corneille Bruck, the president of the state savings bank of Luxembourg, will eventually operate under the name of Coronet.

For Luxembourg, which relies on Radio-Tele-Luxembourg for a substantial fraction of its revenue, the development is something of a surprise. While some observers consider that the move spells an end to the cosy relationship, some say identity, between the government and

At its 1 June meeting, the committee also approved a proposal from Cetus Madison for testing an undisclosed plant variety (the decision was made at a closed session of the committee) and adopted a set of "points to consider" for researchers preparing future proposals for plant field trials. The committee noted that past submissions have often been complete, necessitating their return for additional information. The list of points calls for proposals to include details on specific cultivars, vectors (including documentation that the vectors will not acquire pathogenic potential), data from greenhouse studies and monitoring procedures.

The committee also approved lowering containment of a previously approved toxin-cloning experiment from P4 to P3, or to P2 if the clones prove to be of low toxicity. The proposal, submitted by Dr Alison O'Brien of the Uniformed Services University of the Health Sciences, a part of the Department of Defense, involves cloning a shigella-like toxin in *E. coli*. The aim is the development of a vaccine against dysentery.

Stephen Budiansky

Radio-Tele-Luxembourg, others consider that the government may simply be insuring against the chance that direct broadcasting satellites will be unprofitable.

French uneasiness about the new plan seems to centre on the threat it poses to the commercial viability of the French direct broadcasting satellites. There is also some uneasiness within the European Telecommunications Satellite Organization (EUTELSAT), the European equivalent of INTELSAT. A meeting of the organization, on which national telecommunications authorities, mostly nationalized, are represented, held in Paris towards the end of May, shrank from condemning Luxembourg for a divisive move and instead urged on all its members the need not to take part in competitive international telecommunications services. Luxembourg insists that the plan for Coronet, aimed at delivering broadcast signals only to the operators of cable networks, escapes that criticism and so should be free from constraint.

However this may be, the row between Luxembourg and its larger neighbours has vividly drawn attention to three points. First, Luxembourg is determined to remain a principal exporter of broadcast signals even in the new technological age. Second, national susceptibilities as to who should be free to broadcast to whom are as fierce as they have ever been. Third, the commercial future of direct broadcasting satellites, capable of reaching individual homes, seems to be clouded by Coronet's demonstration that low-powered satellites coupled to cable systems can be built quickly and cheaply. □

Soviet jurisprudence

Nature letter heading for trial

New York

A LETTER from ten Moscow scientists published on *Nature's* correspondence pages in December 1981 (294, 688; 1981) may be cited as nominal defendant in a prosecution under Article 190/1 of the Russian Republic's criminal code, according to a recent Western visitor to Moscow. During the past two years, the signatories remaining in the Soviet Union (two have been allowed to emigrate) have on several occasions been called into the procurator's offices as "witnesses" against the letter. Formally the case is still pending — but the visitor met separately two of the signatories and members of their families all of whom said that pressure was mounting and they feared that formal proceedings might start soon. The plight of one signatory, Dr I.S. Irlin, is referred to in Correspondence (p.490).

The letter drew attention to the signatories' "hard and humiliating" plight since they had filed applications to emigrate to Israel. "Almost all of us have lost our jobs", they wrote, "and we are denied any possibility of continuing with our scientific research. The doors of scientific institutions, laboratories, seminars, conferences and symposia, editorial offices and publishing houses are shut in our faces. We are being deprived of our scientific degrees. . . . We have in fact been thrown out of society, but at the same time are not allowed to leave the Soviet Union."



One of several lines adopted by the Soviet interrogators was the legal fiction that the letter might be a forgery. They accordingly demanded that the signatories give evidence on this point. So far, it appears that all have refused either to confirm or deny the authenticity of their signatures.

The rationale behind the odd piece of jurisprudence of accusing the letter itself and not the signatories is simple — in Soviet law, accused persons have the right to stay mute, but witnesses have no such privileges. Already on at least one occasion, the ploy of "prosecuting" a document has been used. Dr Viktor Brailovskii, the mathematician who for many years

hosted the unofficial Sunday seminars for refusniks and who has just completed a term of Siberian exile under Article 190/1, was originally called as a witness against the *samizdat* journal *Jews in the USSR*, which he edited. During the case new charges were framed against him, and he ended up first in the dock and then in Siberia.

The same ploy may therefore be expected against one or more of the eight remaining signatories (L.A. Dikii, M.I. Freidlin, I.S. Irlin, A.L. Vasilevsky, M.I. Reitman,

S.A. Katz, Ya V. Medvedkov and V.N. Soyfer). The prosecution is clearly keen to get its evidence. The wife of one of the signatories has reported that she has been repeatedly approached by a "friendly" KGB man who urged her that it would be "best" for her husband to testify.

Events seem to follow closely on the trial of Brailovskii. The letter in *Nature* and the accompanying leading article are well known in the Soviet Union. In the circumstances, it seems strange that news of a possible case touching on editorial policy should reach the editor only through the unofficial channel of a returning academic visitor.

Vera Rich

Soviet Jewry

Refusnik flow slows to trickle

Jerusalem

DR Yoram Dinstein, rector of Tel Aviv University, last week announced a new endowment fund to establish university posts for Jewish scientists emigrating from the Soviet Union. The timing was somewhat inauspicious. Israeli universities are so short of funds that six months ago it was feared that the entire higher education system might have to close down altogether. Even now, no vacant posts can be filled, and all non-tenured academics are under threat of redundancy. Positive discrimination in favour of new arrivals from the Soviet Union could be a major cause of friction.

The endowment fund has been set up by Mr Fabian Kolker of Baltimore, Maryland, a governor of the university, as part of his overall strategy to halt the problem of the *nashrim* — Soviet Jews who emigrate on a visa for Israel but who then settle elsewhere, usually in the United States. But the rules of the fund allow the annual interest to be used to help Israeli scientists seeking academic posts should there be no suitable new immigrants from the Soviet Union.

Although Jewish emigration from the Soviet Union is now down to around 50 a month (from a regular 3,500 a month in the early 1970s and a freak 4,500 a month in 1979), the fund has already been used to create a post for one recent arrival, Dr Grigorii Freiman, a mathematician. But under present circumstances it may be some time before another Soviet candidate arrives.

The clampdown on emigration has focused attention once again on the estimated 300 Soviet Jewish scientists who have already filed visa applications but who have been refused permission to leave. Deprived of their scientific posts, "over-qualified" and therefore unemployable in other jobs, refusniks are now liable under new Soviet legislation to arrest for "parasitism" if they are without visible means of support for more than two months.

Since 1964, refusniks have sought to keep up their scientific work through

unofficial seminars and even international conferences, the first of which was totally frustrated — the Soviet would-be participants were temporarily deported from Moscow and invited foreign participants denied visas. Last week, an anniversary conference was held at Tel Aviv University (also on the initiative of Mr Kolker), with scientific papers presented by, among others, Professor B. Pippard, Professor E. Segre, Professor P. Kessler, Professor H. Markovich and the Israeli Minister of Science, Professor Yuval Ne'eman. Former refusniks taking part included Professor Benjamin Levich (a Corresponding Member of the Soviet Academy of Sciences), Dr Eytan Finkelstein, Professor Alexander Voronel who founded the refusnik seminars and Professor Mark Nobel, who succeeded him as convener.

A number of papers by present refusniks were read *in absentia*. The principal aim of such gatherings (including previous conferences in the United States and Stockholm) is to present the refusniks' work in its scientific content and to demonstrate that the seminars (which still continue in Moscow and elsewhere, albeit somewhat less regularly than previously) can generate meaningful work.

The question arises, however, whether such special meetings are the only or the best way of presenting the refusniks' work. Invitations to international conferences abroad are, of course, only symbolic gestures, while conference programmes are now too crowded to permit the custom of the 1960s of keeping blank the time-slot allotted to a Soviet participant who fails to arrive. Even papers sent to be read *in absentia* will usually be crowded out.

One approach not apparently tried so far is to encourage refusniks to submit contributions for poster sessions. Many of the papers at the Tel Aviv conference were, in fact, of poster length, having been dictated by telephone.

With a scientific colleague abroad as sponsor, such participation could prove a valuable way of keeping the refusniks in the world scientific community.

Vera Rich

Sakharov

US academy cancels visit

Washington

MOUNTING anxieties about the health of Dr Andrei Sakharov and his wife Yelena Bonner have forced the US National Academy of Sciences to postpone a planned visit to the Soviet Union next week. Dr Frank Press, president of the National Academy of Sciences, announced last month that he planned to deliver new proposals for resuming formal research links with the Soviet Academy of Sciences. The US academy decided to suspend the links in 1980 as a protest against a Soviet Government decision to condemn Dr Sakharov to internal exile in the town of Gor'kii, some 250 miles east of Moscow.

An academy spokesman said the visit was now in abeyance but the condition of the Sakharovs was being monitored daily. In a telegram sent jointly with the Royal Society of London, the Académie des Sciences de L'Institut de France and the Royal Swedish Academy of Sciences, the US academy at the end of May called on its Soviet counterpart to "act as effectively as possible to help Academician Sakharov and his wife in getting the health care they require and request".

The sudden crisis over the Sakharovs could hardly have come at a worse time for the academy, which had already come under criticism from some quarters for its decision to seek a resumption of ties with the Soviet academy. In an interview on 23 May, for example, Dr George Keyworth, President Reagan's science adviser, said he was "rather surprised" by the decision.

Dr Press has consistently refused to provide details of the proposals he would be taking to Moscow, but apparently hopes to revive the relationships that existed 20 years ago, when distinguished scientists from both countries took part in exchange programmes. Since the US and Soviet academies established a formal exchange programme 25 years ago, 500 scientists have travelled in each direction, but the volume of exchanges dwindled after the US academy declared a moratorium over the Sakharov affair. During 1983, there were a few individual exchanges; 26 Americans visited the Soviet Union for a total of 38 months while 13 Soviet scientists spent a total of 31 months in the United States.

Reaching a new agreement with the Soviet Union may prove difficult even if the political controversy surrounding the Sakharovs abates. Although the US academy is keen to resume links, it wants to change the way Soviet scientists are selected for visits to the United States. US scientists have often complained that under previous exchange agreements, too many junior scientists were being selected by the Soviet Union for visits to the United States.

Peter David

Star wars

Sceptical report from OTA

Washington

THE Reagan Administration has responded with unusual ferocity to a report by Congress's Office of Technology Assessment (OTA) that casts doubt on the ability of the United States to build a space-based "star wars" defence against nuclear attack. Lieutenant General James Abrahamson, former shuttle director and newly-appointed head of the star wars initiative, has told Congress that the report contains "technical errors, unsubstantiated assumptions and conclusions that are inconsistent with the body of the report".

The OTA study, published at the end of April, is the only detailed technical analysis of the star wars idea yet to be published by a neutral government body with full access to classified research data. Its author, Dr Ashton Carter of the Massachusetts Institute of Technology, concluded that the prospect of being able to develop a "near-perfect" defence against nuclear missiles was so remote that it should not form the basis of public policy.

Although they cleared the report for publication, a number of high-ranking defence officials have complained privately that it contained sensitive details of progress in research that should not have been disclosed. A Republican congressman, Henry Hyde of Illinois, has threatened to launch an enquiry into OTA's "alleged security breaches" and to propose legislation making semi-autonomous bodies like OTA "more accountable" in handling sensitive information.

OTA, however, dismisses the allegations of a possible breach of security. Dr Carter, a former DOD analyst specializing in anti-ballistic missile systems, maintains that the report discloses next to nothing about the current state of US research on any of the sensitive technologies. And in a point-by-point rebuttal of General Abrahamson's technical criticisms, OTA insisted last week that its report was technically correct.

Because they are hamstrung by secrecy requirements, neither side in the debate has been able to substantiate its technical criticisms and rebuttals. Much of General Abrahamson's report, for example, consists of simple assertions that OTA based its conclusions on outdated information and was therefore too pessimistic. In response, OTA has been able to say only that it is familiar with all the latest work and that its conclusions are fair.

One of the main technical quarrels is about the use of new fast-burn missiles which, OTA believes, might thwart a star wars defence because their rockets would burn out while they were still in the Earth's atmosphere. The administration's initiative stresses the importance of intercepting missiles during their boost phase, while they can be easily detected by their exhaust plume and before they deploy

their multiple warheads. Fast-burn boosters would be vulnerable for a much shorter time and the atmosphere would help to shield them from several futuristic weapons such as neutral particle beams and X-rays.

General Abrahamson, however, rejects OTA's view that the deployment of such boosters by the Soviet Union could prove a "potent, even decisive" countermeasure against directed energy weapons. It would, he says, take many years and a lot of money for the Soviet Union to deploy a large fraction of its arsenal on such boosters. Even then, their use would reduce the payload and accuracy of their missiles by between 70 and 90 per cent. OTA counters that "nothing like" a reduction of that magnitude would be necessary; it believes fast-burn boosters could be designed to deploy heavy payloads accurately enough to destroy US missile silos, and certainly cities.

OTA and DOD also disagree about how well lasers, X-rays and particle beams could perform as star weapons. General Abrahamson says OTA underestimates the effective range of chemical lasers and disregards promising (but secret) progress in the range of X-ray lasers and neutral particle beams and their ability to penetrate the atmosphere. According to the general, new advances enable a "modest" constellation of beam weapons to "negate most ballistic missile threats". And he dismisses OTA's argument that satellite battle stations and the other space-based paraphernalia of a star wars system would be extremely vulnerable to attack; he says a number of effective means have been developed to ensure that satellites could survive an attack.

General Abrahamson's response to OTA may well have been unusually sharp because it was published before it could be reviewed by the national laboratories most closely involved with star wars research. In subsequent reviews, the general claims, experts at the laboratories found the report full of technical flaws. Even this claim, however, is in dispute; Dr Carter says many scientists at the laboratories support OTA's conclusions.

Whatever its merits or defects, the report has made its mark in Congress. The House of Representatives' subcommittee on international security and scientific affairs cited it in a critical report last month on the administration's space arms control policy. The report said Congress had received no "conclusive" evidence that a perfect or near-perfect defence is technically feasible. It added: "As has been the practice between the superpowers in the nuclear age, the practice of each side developing a countermeasure to the other side's systems could continue unabated under the US and Soviet strategic defence programmes."

Peter David

UK medical research

Neurochemical unit threatened

ONLY a few months after advertising its intention to spend more on research in the neurosciences, the British Medical Research Council (MRC) is taking steps to close one of its better-known units in the field, the MRC Neurochemical Pharmacology Unit at Cambridge. The decision, made known to the staff last week, comes two and a half years after the then director of the unit, Dr L.L. Iversen, announced his appointment as director of a new research laboratory established by Merck, Sharp and Dohme at Hoddesdon in Hertfordshire.

MRC's decision, described as a "decision in principle", will be controversial. The unit has in the past twenty years acquired a reputation for the development and understanding of neuroleptic drugs, chiefly by the refined application of classical pharmacological techniques. More recently, the unit has taken an interest in the histological examination of post-mortem brains, especially from people with schizophrenia.

The unit, one of several maintained by MRC, usually in close association with a university, consists of a score of people of whom perhaps a dozen are established scientists. In its heyday, the unit also played host to large numbers of postdoctoral fellows from elsewhere. The budget for the current year is £375,000.

There is some confusion about the reasons given for closing the unit, said last week to have been made necessary by the difficulty of recruiting a suitable successor to Iversen, at least within the limits of what the council had been prepared to pay for the job. The council said earlier this week that the post had been widely advertised and that the salary offered had been the equivalent of that of a university professor.

On the other hand, Dr Leslie Iversen, the previous director of the Cambridge unit, said that he was "furious" at MRC's decision in principle that the unit should be closed. He said that he had only recently been consulted about at least one eminently suitable candidate who had been interested in the job. He said he planned to write in protest to the secretary of the council, Sir James Gowans, saying that the appointment of his successor had been mishandled and that he doubted whether the council would be able to economize by closing the unit, given that many of the members of the unit were tenured members of the council's staff and that the space vacated by the dispersal of the unit would quickly be occupied by others.

Another factor in the council's decision seems, however, to have been the British Government's refusal of its request for extra funds to sustain an expansion of research in the neurosciences. The council is understood to consider that with recent developments at Imperial College, Lon-

don, Oxford, Edinburgh and Newcastle, it has done as much as could be expected to strengthen the pattern of British research in this field.

A further difficulty seems to have been the changing composition and objectives of the search committee, which at various times seems to have been anxious to confirm the previous pattern of research in the Cambridge unit and at others to wish to branch out in new directions. The search seems to have been in abeyance for a full six months early in the proceedings, when one candidate to whom the job was offered found it hard to make up his mind.

On other occasions, the committee seems inexplicably to have tried to tempt distinguished researchers from the United States with inadequate rewards. The salary offered was formally stated this week to be the equivalent of that of a university professor although it is known that at least to one candidate from overseas figures in the neighbourhood of £30,000 a year were

mentioned and not scorned.

The immediate future of the rump of the Cambridge unit is far from clear. The next step is that a management committee of council scientists will visit Cambridge to hear what plans the remaining scientists may have and eventually to make recommendations to the council.

Dr P.C. Emson said from Cambridge earlier this week that interest among potential postdoctoral fellows in work at the Cambridge laboratory had sharply declined in the past six months. He also said that MRC had only a few months ago given the unit the right to fill a research studentship from the beginning of the coming academic year but had then "taken it away again last week".

Even at this late stage, it seems that at least part of the rump could be reprieved. One possibility that has been suggested is that the remaining members of the unit might be able successfully to persuade MRC to support them with a five-year project grant. Most observers, however, consider that those concerned will quickly be tempted to take posts elsewhere.

John Maddox

High-energy physics

UK debate goes public

THE Kendrew committee set up to review British participation in Europe's high-energy particle physics has now asked members of the research community for their views. The group particularly wants opinions about the relevance and standing of the current UK effort, technical and educational spin-off and of any problems of the European Organization for Nuclear Research (CERN).

Others besides particle physicists may feel moved to respond: the review group will also be pleased to hear about other new areas of science, or grossly underfunded existing areas, that would benefit from a significant increase in support.

The review, under the chairmanship of Sir John Kendrew, was set in motion earlier this year at the instigation of the Advisory Board for the Research Councils, which is studying the question jointly with the Science and Engineering Research Council (SERC). The council expects to spend £52.6 million on high-energy particle physics in 1984-85; £35.6 million of this is the UK subscription to CERN, while most of the remainder relates indirectly to work at CERN. Strong competing claims on the science budget led to questions about whether the high cost — almost one fifth of SERC's expenditure — was justified.

The committee expects to complete its onerous task within a year, and is planning a hectic programme of activity. Apart from the obligatory visit to CERN in Geneva, it will visit other countries to see how their ideas compare with those in Britain. Germany and the United States are provisionally scheduled for visits, and the committee

will also visit British laboratories that work in particle physics.

Legal advice has been sought on the question of Britain's contractual obligations to CERN. It seems to be recognized that Britain could legally withdraw from CERN with one or two years' notice, although the political backlash would be severe. One view is that Britain is morally committed to CERN at least until the completion of LEP 1, the electron-positron collider now under construction and scheduled to start operations in 1987-88.

If, as seems possible, this view is accepted by a majority of the committee, the issues will centre on what should come after LEP. One possibility the committee hopes to look at is that of greater collaboration with the United States — although there are doubts about whether US scientists would be happy to forgo a machine based in the United States for one in Europe.

Speaking at last week's meeting of the American Association for the Advancement of Science, CERN's director general Dr Herwig Schopper suggested that the LEP tunnel could form the basis of a future proton machine with capabilities similar to those of the planned superconducting Super Collider (SSC) but at a much lower cost. The continuing uncertainty over US commitment to the SSC may make it hard for the Kendrew committee to come to any firm conclusions.

Tim Beardsley

Submissions to the review group should be sent to: Dr Keith Root, Room 5/37, Department of Education and Science, Elizabeth House, York Road, London SE1 7PH.

US research grants

New pork-barrel row breaks

Washington

A YEAR of tireless campaigning by university leaders in the United States has failed to keep "pork barrel" politics out of the 1985 science budget. Georgetown University in Washington DC is asking Congress to direct the Department of Defense (DOD) to support a \$220 million energy demonstration project at the university without going through the usual peer review procedures.

The timing of Georgetown's initiative has horrified the scientific community in Washington, which has spent much of the year trying to persuade Congress that research funds should be distributed according to scientific merit determined by peer review. The campaign began last year when two universities, Catholic University in Washington DC and Columbia University in New York, employed a Washington-based lobbying firm to talk Congress into earmarking last-minute funds for scientific facilities at their institutions, although there had been no peer review in either case.

Those developments provoked a stream of resolutions and statements by scientific and university bodies stressing the importance of peer review. Since then, virtually every university spokesman testifying before committees on Capitol Hill has included a statement explaining that wise scientific investment requires that proposals should be assessed on their merits.

Last month, nevertheless, Georgetown's lobbyist, the Reverend Byron Collins, appeared before the House appropriations subcommittee on defence to request \$9 million in 1985 to start work on the university's unsolicited proposal for a coal-gasification and fuel-cell cogeneration programme. The committee's first response has been favourable; last year, it agreed to a Georgetown request to convert \$820,000 of army research funds to planning the project.

Two higher education associations, the Association of American Universities and the National Association of State Universities and Land Grant Colleges, are spearheading a drive to block the Georgetown project. A staff paper circulating within the associations accuses Georgetown of consistent attempts to use its political clout to win research funds. "Over many years", the document says, "the university has taken great advantage of its location to cultivate key members of the Congress and to gain their support for legislation granting the university special treatment . . . Georgetown initiatives are almost uniformly designed to circumvent the established competitive merit-based system for the award of university program funding".

Peter David

Animal legislation

California pounds stay open

Los Angeles

CALIFORNIAN researchers won a major victory last week in their continuing battle with antivivisectionists. A proposed measure to prohibit the use of impounded stray animals in experiments was defeated by 9 votes to 4 in a state assembly committee after intensive lobbying by a coalition of Californian universities and biomedical organizations. The bill, sponsored by state Senator David Roberti of Los Angeles, passed the California senate last year by a healthy margin. It would have prevented animal pounds in California from selling or giving away animals for research and would also have prohibited the transfer of research animals from one state to another.

The bill's defeat is now being interpreted as a major coup for medical researchers throughout the United States. Animal rights groups believe that if such a law had been passed in California, few states would have failed to follow. Nine have already enacted laws that forbid impounded animals from going to research laboratories but researchers in eight of these are allowed to import animals from other states. Only Massachusetts, which passed its animal protection bill several months ago, intends to forbid importation, and as a consequence it is estimated that the costs of experiments using dogs and cats will increase tenfold.

In their successful lobby against the bill, California researchers argued that it would have cost \$12 million to establish breeding facilities for dogs and cats and some \$8.5 million a year to run them. They noted that about 15.5 million unclaimed animals are

killed in California's pounds each year, and that they take only 3 per cent of such animals to laboratories for experiments.

Senator Roberti seems to accept that the bill is dead. He says that he will recommend to humane groups that "the avenue available to them to change the hearts and minds of the legislature is to appeal directly to the people". Senator Roberti said he was not against animal research but, rather, against experiments on pets. But an alternative bill that would prevent pounds from turning "proven" pets over to researchers is languishing in the state senate because few people think it is enforceable.

Meanwhile, animal protection groups in California are livid. "The power of the University of California is overwhelming", said Gretchen Wyler of the Fund for Animals. "They are in control of this state." The next step, she said, will be to go to Washington DC to try to enact federal legislation.

The lobbying success of university and biomedical groups in California was extraordinary. Every major California newspaper supported them, while heart transplant recipients and blind diabetics pleaded before elected officials for continued animal research. Even so, the agony of the decision was clear. Committee chairman Richard Alatorre, as he cast his vote, said that a recent television programme on animal research had been a clear indictment of the academic community and of its conduct of research; but, he said, a good friend who died of leukaemia had begged him to vote for continued research on animals.

Sandra Blakeslee

Raid on US baboon laboratory

A GROUP calling itself the Animal Liberation Front has broken into a laboratory at the University of Pennsylvania, vandalizing equipment worth thousands of dollars and stealing 20 videotapes of experiments on baboons.

The laboratory, directed by Dr Thomas Gennarelli, has conducted experiments with baboons designed to simulate traumatic head injuries to people as part of the university's head injury research programme supported by a \$1 million grant from the National Institute of Neurological and Communicative Disorders and Stroke.

A spokesman for the burglars, who have been identified only as "five members of the Animal Liberation Front", said that Gennarelli's laboratory was chosen for the break-in because his experiments are "notorious" and because of rumours that he had been filming them. Copies of selected tapes provided to local television stations by the burglars show the researchers making jokes about brain-

damaged monkeys, and have provoked predictably hostile public reaction.

Dr Thomas Langfitt, principal investigator of the research programme, defended the as humane and vital for the improvement of the treatment for head injuries. The injuries are induced by accelerating the baboons' heads, without impact. Langfitt said the research has already revealed the mechanism by which damage occurs in such accidents; results have led to mathematical and physical models of the axons and blood vessels in the brain during injury and to the design of improved crash helmets. Langfitt said that the baboons are tranquilized and lightly anaesthetized during the procedure. All the animals are eventually destroyed.

The Animal Liberation Front says it will turn over to the US Department of Agriculture evidence of violations of the Animal Welfare Act. The act requires the proper care and housing of animals, but leaves experimental design entirely up to the researcher.

Stephen Budiansky

Forestry

Taxing changes in prospect

POLICY proposals published this week by Britain's Forestry Commission include for the first time special provisions for the protection of Britain's ancient semi-natural woodland. In a consultative paper, *Broadleaves in Britain*, the commission suggests measures to minimize loss of all broadleaved woods and introduces guidelines agreed with the Nature Conservancy Council (NCC) for managing ancient semi-natural woodland. This change of heart by the commission, which previously failed to recognize this category at all, is seen as a major concession to conservation interests.

According to a survey (as yet incomplete) by NCC, losses of ancient semi-natural woodland over the past 40–80 years in different counties range from 10 per cent to over 60 per cent, with most counties so far surveyed showing losses of over 40 per cent. The commission, which advises private foresters and manages its own forests, now recognizes a good case for slowing the rate of future loss, and proposes new management guidelines that will automatically check felling proposals and grant applications against the NCC register of ancient woodland. For woods so categorized there will be "a presumption in favour of maintaining the species already present" and owners will be persuaded to adopt methods of working that are "least disruptive of the woodland habitat". Broadleaved woodland in general should be maintained at about its present extent by requiring replanting with broadleaves after felling and allowing permanent clearing "only exceptionally".

The Forestry Commission has been under intense pressure from conservation groups to change its policies in this area, and has consulted extensively with interested parties for more than two years. There was therefore some surprise this week that the new document is consultative and not a definitive policy statement. Comments can be made on the proposals even at this stage. The commission hopes nevertheless to offer its final advice to government before the end of the year, as proposed tax changes and higher grants for establishing broadleaved woodlands will need ministerial approval.

Most of the broadleaved woodland in Britain is in private hands. Timber Growers UK Ltd, which represents foresters' interests, welcomes the Forestry Commission's proposals as a big step in the right direction. But, in the view of the association's chief executive, Mr Ronnie Williams, the commission could have been much firmer. Private foresters are keenly aware of the need to improve financial provisions for owners of broadleaved woodland, who are at a disadvantage compared with owners of the faster-growing and more profitable conifer stands. The Forestry Commission now proposes to

make it much easier for owners of mixed estates to claim tax relief on slow growing broadleaved plantations, a move welcomed by the timber growers. But, they say, the commission does not go far enough. Only by making broadleaved woodland management less of a financial burden in the hundred years or more from plantation to financial returns will the pressure to destroy ancient woodland be removed. It is also pointed out that the commission has hardly been providing a good example by its own planting policies: the vast majority of recent Forestry Commission plantations are of conifers.

The Forestry Commission makes much of the fact that the total area of broadleaves in Britain is now 15 per cent greater than in the 1940s, and its proposals could be briefly summed up by saying they aim to maintain the *status quo*. Private foresters, however, argue that a million acres of woodland were

lost in two world wars and want a return to the levels of the turn of the century. But at present there are severe economic obstacles to broadleaved production, mainly the high costs of establishments, the low productivity and long rotations.

The Forestry Commission is currently reconsidering much of its research activity, and that on productivity of broadleaved species is one important area of discussion. The new *Broadleaves in Britain* document gives some indications of where it might be heading. The review group that produced the document considers it is "doubtful" whether improvements in growth rate can be achieved through the use of fertilizers, and in the long run the improvements will come about through genetics, most probably using vegetative propagation techniques and clonal testing. There is also, according to the group, a need for more information on total biomass production and how this is affected by spacing and harvesting regimes, as well as the properties of species mixtures and newly introduced species.

Tim Beardsley

European biotechnology

Optimism abated as summit looms

Brussels

OFFICIALS of the European Commission fear that the Community's proposed biotechnology programme is falling foul of the present crisis in the Community's affairs. One sign of this is that the powerful agricultural lobby is mobilizing to block certain reforms that would have helped the growth of a strong biotechnology industry.

In spite of the generally favourable reception of the proposed programme by both research and industry ministers in November and December last year, that conjunction of omens seems now to be dissipating. "The alliance is coming unstuck", complained one official, citing the objections now raised by the sugar beet industry to a new pricing regime for the industrial use of sugar. "The sugar beet industry thinks that the biotechnology programme is the thin end of a wedge" leading to lower prices which, the official guesses, is the reason why agricultural ministers are now dragging their feet.

The Commission, meanwhile, is trimming its optimism about biotechnology to the changing wind. Last year, the Commission asked for a five-year programme costing 200 million European Currency Units (ECU) (about £118 million) but in its revised proposal put forward on 26 April, the Commission asked for only 88 million ECU covering only two of six of the original proposals — research and training and "concertation" with other biotechnology programmes.

At this level, the programme would represent a little more than a million dollars per country per year, an order of magnitude less than the Esprit information technology programme. The Netherlands

and Denmark are unhappy about the size of even this proposal, while the United Kingdom and West Germany want the programme cut back drastically. "The council seems intent on reducing the whole thing to ashes before they have to build the whole edifice again", said one frustrated scientist.

The cuts and delays are especially galling for officials still smarting from the criticism earlier this year from the US Office of Technology Assessment that Europeans are not aggressive in commercializing biotechnology and pose little threat to the US industries.

At the twice postponed meeting of science ministers, now fixed for 29 June, there is even a danger that discussion of biotechnology will be swamped by two other items of high importance — telecommunications and the fusion programme. A spokesman for Research Commissioner Etienne Davignon insists that biotechnology retains its high priority, but British officials complain that they have yet to see where it fits into the Commission's larger scheme of things.

A further imponderable is that, by the end of June, the next Community summit at Fontainebleau may or may not have resolved the Community's budgetary crisis. The Commission may decide to go for a decision in principle on biotechnology, letting the question of funds hang loose until later, but in any case the programme will have to await an opinion of the newly elected European Parliament, and so is not likely to see the light of day before next year. "But if things go badly at Fontainebleau", said one senior European official, "it could be a catastrophe for European research".

David Price

Federal laboratories

Packard calls for flexibility

New York

IMPORTANT changes in pay and conditions may be on the horizon for scientists employed in the United States' 700 federal laboratories, which include the National Institutes of Health and laboratories run by the Departments of Energy, Defense and Agriculture. An interdepartmental committee headed by the White House science office has drafted legislation to free the laboratories from civil service pay regulations so that they can better compete with the universities and industry for talented scientists and engineers.

Addressing last week's meeting of the American Association for the Advancement of Science (AAAS), Mr David Packard, chairman of California's Hewlett-Packard Corporation, said the model legislation was proposed in a draft report to be submitted to President Reagan in July. But he predicted that the legislation would stir up considerable opposition from vested interests in the civil service.

Mr Packard, a personal friend of President Reagan, strongly criticized the civil service's inflexible procedures for pay and promotion in a review of the federal laboratories he conducted last year for the White House Science Council. The new legislation, he said, would create a separate personnel system for scientists and technicians, replace narrow pay bands with broad ones, waive the existing pay ceiling for senior employees and allow promotions to take account of merit as well as seniority.

The report going to President Reagan in July will claim there has already been impressive progress towards implementing other recommendations in last year's Packard report. In response to a call for clearer definitions of the missions of each of the national laboratories, for example, the Agricultural Research Service has for the first time drawn up long-term research plans for its institutions. And the Department of Energy has begun to make its laboratories produce five-year plans in which each institution must state its direction, role, expected changes and budgets.

But the director of the Oak Ridge National Laboratory, Dr Herman Postma, told AAAS that progress had been uneven. Both Congress and the Office of Management and Budget (OMB) continued to block a proposal to bring financial stability to the laboratories by setting rolling budgets for two or three years at a time. The Department of Energy, responding to Packard and earlier reports, had begun to give each laboratory an "exploratory budget" to be spent at its director's discretion, but the level was between half and one per cent of the laboratories' budgets instead of the 5 to 10 per cent recommended by Packard.

Peter David

Antarctic treaty

Minnows smell a rat

Tokyo

THE Antarctic Treaty Consultative Parties (ATCP) ended their secret deliberations in Tokyo on 31 May with the announcement that "good progress" had been made towards an agreement permitting the exploitation of Antarctic mineral resources. Representatives of nations that have no say in how Antarctica is to be carved up, as well as conservation groups that had come to Tokyo to monitor the meeting, took a less charitable view.

Of the sixteen members of the Antarctic Treaty, seven have territorial claims to huge areas of the continent which have been held in abeyance since the treaty was signed in 1959. Entry now is restricted to those who carry out a full programme of research in the Antarctic. Despite the very great cost of maintenance of bases and ice breakers capable of reaching them, both Brazil and India thought it worthwhile to join last year (see *Nature* 307, 105; 1984).

Under discussion in Tokyo, at the Fifth Special Mineral Resources Meeting, was a plan put forward by New Zealand representative Chris Beeby. The meeting was surrounded by secrecy — despite repeated protests from the Netherlands, not even representatives of the fifteen nations that have signed the Antarctic Treaty but which do not carry out research there (the "Non-Consultative Group") were allowed to send observers. But copies of the original draft of the Beeby plan were circulated by conservation groups, and individual delegates were willing to discuss privately amendments made since the last meeting.

Essentially, the plan proposes that mineral exploitation be regulated by three types of institution. At the top is a central commission which will be the ultimate political body for the whole mineral regime, with membership restricted to the 16 ATCP members and to nations with definite plans for mineral exploitation. Next will come a scientific and advisory committee whose members will be appointed by the central commission, to provide "technical guidelines" including those for environmental protection. It will not, however, have any power to enforce these guidelines.

Real power will reside with "regulatory committees", one for each area of the Antarctic when a proposal is made for exploitation in that region. Each will contain representatives from the United States, the Soviet Union, four nations with territorial claims, the nation proposing mineral development and another nation nominated by it.

What this plan neatly achieves is an accommodation between those with territorial claims and nations that actually want to carry out mining operations in the Antarctic. Beeby speaks proudly of the great success that the Antarctic Treaty has

achieved over the past twenty years in keeping international rivalries out of Antarctica. His plan is primarily concerned with maintaining this internationalist spirit while permitting mineral exploitation to take place.

But to the smaller nations the whole plan seems designed to keep power in the hands of the Antarctic Treaty members. Malaysia received overwhelming support from the last meeting of the non-aligned nations for a resolution calling for a UN debate on the Antarctic issue during 1984, a move that could end with a conference like that of the Law of the Sea which would call Antarctic resources, like ocean bed resources, the heritage of all mankind.

The non-aligned group accuses the treaty members of trying to tie up a minerals agreement to pre-empt such a move. Indeed, if environmental groups are right, preliminary investigations of Antarctic oil resources have already begun — despite a 1977 agreement to restrain exploration while "satisfactory progress" is being made towards a minerals agreement.

What disturbs the environmentalists more, however, is that there would be no publicly accessible central body with the power to ensure that the Antarctic environment is not damaged under the Beeby plan. To divide the Antarctic into regions, each under the control of an eight-nation group with an interest in exploitation, would, they claim, prevent any overall or consistent ecosystem management. And the Beeby plan contains no provision to regulate preliminary prospecting, even though a test drilling that went wrong could have appalling effects. There can be no guarantee that a nation will effectively discharge its obligation to supervise the mining operations of its nationals. Instead, the major conservation group concerned, the Antarctic and Southern Ocean coalition of 150 environmental organizations from 37 countries, is calling for an Antarctic Environmental Protection Agency to oversee all Antarctic operations. There is sympathy for this idea from some ATCP members, but not from the two superpowers.

That the Beeby plan has only been slightly modified since the last meeting of the Mineral Resources Group suggests that its form is close to being acceptable by the ATCP members and that the idea of an Environmental Protection Agency will stand little chance — although it is impossible to be sure, given the secrecy of the discussions. Discussions will continue in February 1985 with Brazil as the host country. There, for the first time, the non-consultative parties to the Antarctic Treaty will be allowed to send observers and it may be possible for more than a handful of nations to find out what is going to happen in Antarctica.

Alun Anderson

Pleas for would-be emigrés

SIR—We wish to draw your attention to the case of the respected Soviet cancer researcher, Dr Iosef Irlin, who has been involved in the fields of tumour virology and tumour immunology. His work has been of theoretical importance and in no way related to matters falling within any national security interests.

Dr Irlin applied four years ago for permission to emigrate with his family to Israel, to be reunited with other members of his family there, and he has reapplied repeatedly during the past four years. All his requests have been refused, without explanation. During this time, he has been without professional employment and his several applications for jobs have been rejected.

In an earlier letter, Dr Irlin states:

It is now four years that I have been in refusal. As you must know well, being engaged in my profession, I have never been connected with any state secrets or security, dealing only with experimental oncovirology and immunology of experimental tumours — quite an open branch of science. In spite of this, my family and I were not allowed to emigrate to Israel and none of the officials, despite my numerous applications, took the trouble to give me any good ground for the refusal. By now, I repeat, for four years, I have been without a professional job and practically without hope. All my efforts to get any decent job have failed.

We appeal to scientists from all countries, including our Soviet colleagues, to use all their influence so that Dr Irlin, together with his family, is allowed to leave the Soviet Union to live and work in the country where he and his family wish to stay.

GEORGE KLEIN

JERZY EINHORN

Karolinska Hospital, Stockholm, Sweden

STUART A. AARONSON

National Cancer Institute, Bethesda, Maryland, USA

FRED RAPP

Pennsylvania State University, Hershey, Pennsylvania, USA

ANDRÉ LWOFF

Pasteur Institute, Paris, France

THOMAS GRAF

European Molecular Biology Laboratory, Heidelberg, FRG

DAVID BALTIMORE

Massachusetts Institute of Technology, Cambridge, Massachusetts, USA

ROGER WEIL

University of Geneva, Switzerland

SIR—I wish to support in the strongest possible way the appeal of Dr André Lwoff, and other Western biochemists and geneticists on behalf of Dr David Goldfarb whose exit visa for Israel has recently been withdrawn by the KGB (see *Nature* 308, 679, 766 and 309, 104; 1984).

I have known and admired Dr Goldfarb

since he came to work in my microbial genetics unit at Hammersmith Hospital, London, some twenty years ago, and respected him as among the most honest, able and innovative Soviet geneticists of the post-Lysenko era. To those of us who have known Dr Goldfarb well, he has always reflected credit on the Soviet Union for his work on microbial genetics. I therefore found his harassment by the KGB when he applied for an exit visa in 1979 not only unjust but incomprehensible, especially in view of the assertion by the Soviet Academy of Sciences that he knew no state secrets. He also demonstrated his courage and patriotism at the siege of Stalingrad where he lost a leg. The latest withdrawal of his visa, again approved by the Academy of Sciences, on the eve of his departure for Israel savours of paranoid persecution and causes great distress to his many friends abroad in view of his advancing years and poor health. I therefore hope, in the interests of all those who support goodwill and international collaboration in science, that Goldfarb's case will shortly be resolved in his favour.

WILLIAM HAYES

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Skew physics

SIR—I was struck by the remarks on Fritz London in your recent article (*Nature* 10 May, p.109). Amongst other qualities, London was gifted with modes of apprehension of physical problems that were skew, or at any rate out of line with those of his contemporaries, with the consequence that some of his publications at first lacked the full impact their importance warranted. As some compensation, his originality enabled him to grasp more confidently skew developments presented to him.

I was fortunate to have personal experience of this trait in relation to my earlier letter (*Nature* 132, 1002; 1933) on the change from "aromatic" to "metallic" electrons in organic compounds. This text was written with some unease because of its novelty, but on my walking back from posting it to your offices I was introduced to Fritz London by F.A. Lindemann, who was chatting with him at the entrance to the Old Clarendon Laboratory at Oxford. Rather tremulously, I showed them my letter. Lindemann read this politely, and said little, but Fritz London immediately grasped what it was trying to focus on about the unusual physical properties of aromatic molecules, such as their large diamagnetism, and reassured me. He himself later developed this theme more fully.

His kind of responsiveness raises the interesting educational problem of whether

teachers may be justified in describing select skew approaches to physics to their pupils, not only to leave loose ends for innovative developments, but generally. It can be argued that the best informed financial patronage for research, and most valuable support for research programmes, is to be sought from managers and directors who understand a mixed conceptual language much more readily than would physicists with much stricter training in scientific communication.

A.R. UBBELOHDE

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Sea as dark as wine

SIR—When Homer sang of the "wine-dark" sea (see *Nature* 303, 568, 1983; 307, 590; 309, 204, 1984) what he had in mind must surely have been its darkness, rather than the colour, and "dark as wine" might be a better translation. After all, one can say that "her eyes sparkled like diamonds", without implying that they were of the same colour.

Ionian Greeks knew all about wine, but with what other fluids would they have been familiar, to be compared in darkness with the sea? Blood would not have done, and the Homeric heroes had no use for ink, which was a muddy concoction available, if at all, only to scribes. So, gazing into the mysterious depths of a calm dark sea, how better could the poet then have described it, than with his simile of the transparent darkness of a full cup of wine?

C.B. GOODHART

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Luddites defended

SIR—It has become fashionable to describe negative attitudes to technological change by making an analogy to the Luddites. Terms such as "neo-Luddite" or even "latter-day Luddite" are appearing regularly. The most recent I have noted is in David Sills' review (*Nature* 10 May, p.185). p.185).

The term seems to imply a senseless denigration of new ideas, an attempt to retain the old. I would suggest that this is a gross generalization. It strikes me that the original Luddite leaders were more concerned with the terrifying effects that new technology was to have on the workforce. Whilst their methods of protecting people from unemployment, with its associated evils, were violent and destructive, their intentions were highly honourable and deserving of our attention today.

RICHARD MORRISH

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Contrasts in near-similarity

The Low Countries, Belgium, Luxembourg and the Netherlands, are far from being the homogeneous corner of Northern Europe that geography suggests.

THE hallmark of densely populated Europe is diversity; the Low Countries — the Gallic name for the regions bordering the estuary of the Rhine — are Europe in microcosm. Socially and politically, the Netherlands, Belgium and Luxembourg between them embody all the features of a continent repeatedly reworked by twenty-three centuries of recorded history. Since the Roman pacification of northern Europe, the strategic importance of this patch of land has given it the unenviable distinction of being that on which the great European armed conflicts have been staged. The marks of those battles are still plain to see in every other rebuilt market square. The wonder is that so much remains of the distant past — cathedral spires glimpsed from miles away over flat alluvial farmland, the art treasures of many centuries and the indomitably cosmopolitan conviction that the whole of Europe can be run from the back yards of these northern fens.

The following pages will also show the Low Countries to be in microcosm representative of Europe's contemporary problems. Once comfortably prosperous economies are now, surprisingly, less so. Communal divisions rooted in language or religion are now openly contentious, with the result that tiny nation states are being further splintered into autonomous regions. Fierce public arguments rage (especially in the radical Netherlands) on questions such as the safety of nuclear power stations or the prudence of providing hospitality for other people's nuclear missiles. (Last week, the Dutch coalition government postponed for another eighteen months the decision whether to carry through its 1979 obligation to provide sites for nuclear missiles of the North Atlantic Treaty Organization.) And Belgium and the Netherlands must approach Europe's contemporary dilemma of how to join the new high-tech industrial revolution while keeping public expenditure to a minimum.

That Belgium and the Netherlands should be faced with such different problems is not surprising to Europeans, but may catch others unawares. In reality, the two countries have little more in common than their place on the map. (Luxembourg is something else again.) Historically, the Netherlands has the greater claim on public attention, perhaps because so many Dutchmen were quick to recognize the importance of what was happening in southern

Science in the Low Countries

THIS survey of science in Belgium, Luxembourg and the Netherlands has been compiled and written by Robert Walgate, Chief European Correspondent of *Nature*.

Europe during the Renaissance. Erasmus, part-philosopher and part pre-Newtonian physicist, was one of them. In the seventeenth century, van Leeuwenhoek did for the microscope what Galileo had done for the telescope, showing the potential of primitive pieces of glass. Huygens, whose physical insight made wave propagation through a medium a branch of mechanics, was a Dutchman. It remains something of a miracle that the University of Leiden should have served as a durable bridge between nineteenth-century physics and what came later through the work of H.A. Lorentz and his successors, notably Kramers. It is understandable that the government of the Netherlands should now be moving with great caution to impose external direction on the patterns of research in universities which have so recently been able to do so well.

Industrially, the two countries are also sharply different from each other. Mainland Europe's late response to the industrial revolution of the nineteenth century prompted quite different responses in the two small northern states. Both have made their way in the world by being industrious, but in very different ways. The Dutch seem to have concluded that it would be uneconomic just to make electric light bulbs simply for themselves and so Philips has made light bulbs for half of Europe. Now there are Shell and Unilever as well, all of them enterprises based squarely on a mercantile flair and the self-conscious application of research and development to the manufacture of products that people elsewhere will buy. Is this a continuation into the present of the old Dutch willingness to travel abroad or a measure of the strength of the Dutch universities? Belgian industry has taken a different route. Until quite recently, Belgium has been to northern Europe what Birmingham used to be to England, a conglomeration of small-scale metal (and diamond) workshops. (But now there are international pharmaceutical companies, as well as other people's multinationals such as I.T.T.) Is it that Belgium has had only modest expectations of what its universities can accomplish for its industry,

at least until comparatively recently?

Such questions prompt over-simple answers. Since the Second World War, and with the unwinding of overseas empires in both Belgium and the Netherlands, it has been plain that each of them can function competitively only as a part of a larger European enterprise. It is therefore no accident that the Benelux economic union should have been a precursor of the European Communities. Each of them now gives outsiders the impression that it is marking time, waiting for the international climate to change for the better. The Dutch, meanwhile, have been flexible and ingenious in hitching some of their academic science to international enterprises, especially in fields such as astronomy. Belgium, with its language problem, is by contrast being forced along a path — separate pots of public money for separate linguistic regions — that cannot but be cramping for Belgian universities and, ultimately, for Belgian science. Can the mutual suspicion of the Walloons and Flemish be that important to them both?

So, it seems, the years immediately ahead are bound to be crucial for all three of the Low Countries. Will progress towards European integration make it easier for them to concentrate intellectual resources, and research and development in particular, on a smaller range of objectives without at the same time running the risk of neglecting activities that turn out, in the course of time, to be even more important? This, the recent dilemma of most European states in science and technology, is necessarily more acute in Belgium and the Netherlands than elsewhere. The Netherlands, with its established international connections, is better placed than Belgium, now bent on modernizing its metal-working industries and everything else in sight. Both countries would, however, benefit enormously from the more thorough integration of European academic institutions, universities and basic research establishments. Indeed, the problems encountered by Belgium and the Netherlands are a pointer not merely to a potentially fruitful area consistently neglected by European politicians (which will become more important as other states accede to the European Communities) but also to the way in which the rest of Europe does not fully share the benefit of the intellectual resource of these northern states. □

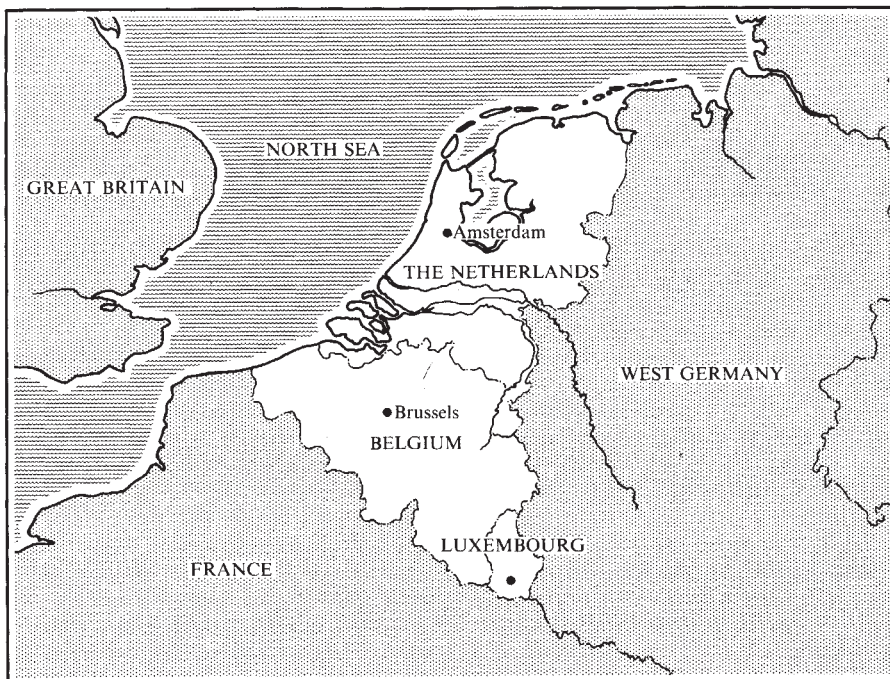
Geography

Small lands, big industry

ARE the Low Countries small? Some 14.3 million people live in the Netherlands; 9.9 million in Belgium; and 365,000 in Luxembourg: a total of a little more than 24 million people, less than half the population of Great Britain, France, Germany or Italy, and a tenth that of the United States. But their joint population density is three times that of France next door. And these people in their cabbage patches are highly productive: the Netherlands has probably the highest ratio of gross national product per square kilometre (£2.4 million) in the world.

Moreover, in the small continent of Europe, the Netherlands can claim to be medium-scale: politically, it chooses either to trail the big countries or lead the small, according to the policy it is pursuing. Smaller Belgium is further divided, roughly in half, by the constant regional sniping between Dutch-speaking Flanders in the north and French-speaking Wallonia in the south. Luxembourg, of course, is tiny, a little anomaly in modern Europe.

Geographically most of the Netherlands and the northern half of Belgium form the delta of the great river system of the Rhine, the Meuse and the Escaut rivers; and nearly half of the Netherlands (and more than half its population) is below sea-level (the windmills were — and are — water pumps, not flour mills). This gives both countries good flat agricultural land, and the Dutch, in particular, are renowned for their farming and agricultural science.



One Dutch university, Wageningen, is devoted to agriculture. Apart from that, the Netherlands has nine full universities, and three technical (engineering) universities. Three of the universities have religious affiliations: the Free University of Amsterdam (Dutch Reformed) and the Catholic Universities of Nijmegen and Tilburg.

Apart from agriculture and a strong dose of Calvinism (now forgotten, to judge by the drugs and flesh-pots of Amsterdam), in the past the Dutch have relied on sea-trade (which gives them an affinity with the English), and good business sense; but they also manufacture. In fact Holland supplies

much of General Motor's steel (sea transport to Detroit up the St Lawrence is "almost free", they say). The Dutch also boast the headquarters of the multinationals Philips, Shell and Unilever, and are strong in the fermentation and pharmaceuticals business. Since the 1960s they have enjoyed a bountiful supply of natural gas from Groningen in the north of the country, which some say has distorted the economy and the spirit of the Dutch.

Belgium has coal in Wallonia, and hence steel and the chemical industry — dominated by Solvay (which supported the historic Solvay conferences in physics). Flanders developed on textiles — for centuries it made cloth from British wool, and Belgian lace is famous — but it also has the almost independent offshoot of Bell Telephones, Bell Antwerp, flourishing foreign investment and is turning rapidly to new industries. It is thus outpacing the heavy industry of Wallonia, a circumstance which has now become a major cause of regional friction.

Belgium has six universities (three Flemish and three French) and eleven regional "university institutes". Two of the universities are Catholic (Leuven and Louvain-la-Neuve) and two free, that is neither fully state-controlled nor religious (the Flemish and French free universities of Brussels).

Luxembourg, finally, in the far south and hillier part of the region, has been predominantly a steel and metals town (half of its industrial employment was in steel 10 years ago, although the fraction is now declining and steel production with it.) After steel, Luxembourg has rubber (Goodyear's European laboratory is there) and banking (it is like a little Switzerland to rich Belgians) and is now developing new industries. It has the first year of a university, and something approaching an academic hospital.

Charlemagne's fondness for bathing

THE Netherlands, Belgium and Luxembourg are the northern tip of a corridor of states that once separated the Germanic from the French empires; and as rebellious buffer states, they have often been warred over — or upon. (It is appropriate, said one sardonic Belgian, that the North Atlantic Treaty Organization NATO has made its headquarters in Brussels.)

It is believable, although not necessarily true, that the blood soaked countries owe their existence to Charlemagne's love of a hot bath. This last great unifier of Europe was crowned in AD 800 and made his court at Aachen, now a German city but just over the border from the southern Netherlands. He chose that city, it is said, because as a lover of the good life he liked to bathe in its hot springs — there may have been some sound military reasons too, as Charlemagne was a soldier as well as a lover of baths, but these reasons are unclear. But for whatever cause, Aachen was the focus of his empire when he was planning its division among his sons, and Charlemagne

seems to have planned the division symmetrically.

But even Charlemagne's plans could come adrift, and in the event, only one of his sons survived, leaving the empire intact for a generation. That son then enacted the division among his own three sons: one took the Germanic lands, so providing the early foundation of Germany; one took what is now France; and one was granted a corridor of land which ran from the low countries south through Alsace-Lorraine (now part of France) and on to the Mediterranean. These were the buffer states between France and Germany, of which the low countries were for long the marshy and neglected north.

The present states were first defined when Belgium seceded in 1830 from the United Netherlands (a product of the Napoleonic wars) after a revolution in Brussels; and second when Luxembourg won its independence from the Netherlands in 1890. In royal terms, they now form two kingdoms and a duchy (Luxembourg). □

Economics

The end of equality

ONE of Belgium's grander scientists, Nobel Prize winner Ilya Prigogine, is concerned — among other things — with a topic that proves strangely appropriate for this review of science politics in the Netherlands, Belgium and Luxembourg: the matter of "strange attractors".

These are states in phase space towards which complicated systems may converge, somewhat like a ground state in classical physics.

So Belgian and Dutch science, it seems — to leave Luxembourg aside for the moment — have converged along quite different paths towards the same strange attractor. Moreover it is one that seems to afflict much of European science: a kind of balkanization of science politics that ends up distributing government resources more or less evenly, or at least much more evenly than is good for the health of research — which, with some exceptions and caveats, often depends on the very inequitable principle of the best getting the most.

But "equitable distribution" has developed to an extraordinary degree in the Netherlands, where until very recently only 7 per cent of basic research funds in the universities went through a semblance of national peer review and where, of that 7 per cent, perhaps only a quarter went through a really serious competition for funds. Despite government attempts to introduce selection, and hence a kind of scientific aristocracy, as recently as last November a tongue-in-cheek science policy official said "we don't like to make kings and queens here". This was not treason against the Dutch Royal family: he was drawing attention to another honoured Dutch tradition, even distribution — a belief in a good solid mean value rather than peaks and troughs.

This Dutch situation seems to stem, perhaps as does much of the Dutch character, from centuries of cooperation to drain the land and keep out the sea; and it was emphasized by the democratic revolution in the universities in 1968. But it can also be seen as a Dutch version of a general European principle: that academics receive funds in proportion to their academic-political weight. (Perhaps that in turn stems from the long pre-scientific history of academic bickering in Europe.) It is evident throughout Europe that there are academic kings, and that — more than is the case in, say, the United States — kingship does not necessarily correspond to ability or productivity. In the Netherlands, this principle has merely been reversed. In the Netherlands, university democracy — and the very fear of kingship — has merely spread the king's rights over the whole academic community. This then taxes the government

Table 1 Gross national product (GNP) and population

	Working population (million)	Area (10 ³ km ²)	GNP (\$ thousand million)	GNP per head (\$)	GNP per km ² (\$ million)
Netherlands	5.0	41	106	23,000	2.6
Belgium	4.1	31	79	21,000	2.5
Luxembourg	0.16	2.6	4	25,000	1.5
United Kingdom	26	244	400	15,000	1.6

These figures demonstrate the relative economic weakness within the low countries of Belgium, in terms of per capita GNP, but the strength of the whole region relative to the United Kingdom.

Table 2 National research and development budgets

	Government			Industry	
	Total (\$ million)	% GNP	Defence component	Total (\$ million)	% GNP
Netherlands	1,007	0.95	3%	986	0.93
Belgium	333	0.42	0.3%	698	0.93
United Kingdom	3,821	0.95	50%	3,423	0.86

This table again shows a relatively poor performance by Belgium — in terms of government support as a fraction of GNP, which as Table 1 shows was itself low relative to the Netherlands. Allowing for the small fraction of the low countries' spending on defence science, the Dutch research and development budget fraction works out somewhat higher than expected in an advanced country, and the Belgian slightly lower. There are no official science budget figures for Luxembourg, but an unofficial estimate puts government research and development at a miniscule 0.1 per cent of the Luxembourg GNP: most of the research there is applied research done by the steel and rubber industries.

Table 3 Division of government research and development budget

	Applied research in industry	Applied research in agriculture	Fundamental research	Human and social sciences
Netherlands	14%	8%	56%	22%
Belgium	35%	6%	41%	18%
United Kingdom	33%	8%	47%	12%

This indicates the low weight given to industrial research support by the Dutch government, and the relatively high weight given to the social and human sciences in both Belgium and the Netherlands. In the Netherlands at least, these sciences have been adopted enthusiastically by government, the administration, and politicians as an aid to policy-making. And the Dutch neglect of industry in fact reflects the great weight of the so-called "big five" companies in the Netherlands — including Philips, Shell and Unilever — which between them cover 70 per cent of Dutch industrial research and development. The big five have been thought, no doubt correctly, well-able to look after themselves. The neglect, therefore, has been of Dutch small and medium size industry; but the government has made and is making moves to correct the imbalance.

Table 4 University research funds

	University students	University research funds (\$ million)	Proportion through research councils	University research as % of all national research and development	University research funds per student taught (\$)
Netherlands	147,000	522	7%	25%	3,550
Belgium	110,000	223	27%	21%	2,030
United Kingdom	251,990	906	46.2%	11%	3,595

Here we calculate the research funds available (including the research proportion of academic salaries) per student taught — a figure which gives a measure of the research intensity of the universities compared with teaching. On this table the Dutch universities have nearly double the research intensity of the Belgian. This makes the Belgian universities look poor, but academics in Holland admit that their universities have been among the richest in the world. Other figures of interest: the small fraction of Dutch research funds that goes through research council peer-review, and in both countries the high proportion of university research as a fraction of all research and development (about double the fraction in the United Kingdom). These figures are based on the latest OECD science and technology indicators (1984) which refer to the year 1979. The figure of 46.2% for the proportion of UK university research sponsored by resource councils relates to 1980/81.

proportionally, in a kind of royal brotherhood.

The problem is only slightly easier in Belgium, where funds have been distributed more unevenly, and to some extent on scientific grounds — particularly since the institution of a prime-ministerial “top-groups” fund in 1970. However, a large fraction of funds has been distributed among the nominally six complete universities according to what Belgians describe as “the theory of the four forces” — equal distribution between Catholic and non-Catholic, and between Flemish (the Dutch-speaking northerners) and Walloon (French-speaking southerners).

As a result, then, professors and researchers in both countries, in bulk and on the average, have received their money more through mere queuing, more through the essentially political demand of “fair proportion” than through scientific judgement.

However, Belgium and the Netherlands are not entirely without systems of selective

funding, and the occasional watering of the best plants can and does have dramatic effects: as, for just two examples, with Prigogine’s own laboratory, and astronomy in the Netherlands.

Moreover — and hence the headline above — things are changing in the Low Countries. The well-worn “economic crisis” is forcing drastic reappraisal of the equal-shares approach and, with very different mechanisms and effects, selectivity is increasing. The Netherlands is making the closest approach to extending peer-judgement in the basic sciences; Belgium is failing in this, and at the same time reducing its support of basic science. Both countries are increasing judgement at the level of national and — in the case of Belgium — regional politics. In this way, applied scientific and political criteria are encroaching very fast on the Belgian universities, faster than is probably advisable. These different approaches and their consequent problems are outlined in the following pages. □

The Netherlands

Shadow boxing with peer review

THE most fundamental change facing researchers in Dutch universities is not a loss of funds — though they face that too — but an increase in national refereeing of what funds there are. This threatens (or promises, according to your point of view) to change the whole basis of support for the bulk of basic research in Holland — and to put a new policy instrument in the hands of the government.

Until recently, over 90 per cent of the research funds received by the universities underwent no national review. The money was — and much of it still is — distributed at university level, by local research councils. These, with some exceptions, such as the Leiden council which has developed a method of selection based on citation analysis, find it difficult to make negative judgements about colleagues’ work. And even at Leiden, such judgements are painful: because citation analysis can be challenged; because council members are judging the work of friends met daily; and because — given the small scale of a single university — often the work to be judged is in a discipline far from entirely understood by most council members. As a result, of course, money has been largely distributed through a system of courtesy rather than quality.

This may be gentlemanly; it may allow some rare lines of research to be followed that would otherwise be forgotten; but it leads to a loss of competitive edge, and it certainly does not suit times when money is short.

Hence the introduction of a system called “conditional financing”, which offers a gold star — “protection” — to research projects that gain approval by national committees.

But for the moment, although many gold stars have already been awarded, no one really knows what this approval — or the lack of it — will mean. For the time being, the money comes to all much as before: the scheme has been one merely of labelling, the marking of a cross on the door. It is the time, in Dutch research, of the phoney war.

However, protection, says the minister of education and science Wim Deetman, does mean that a project will be exempt from further unforeseen cuts in university spending for five years. (Or at least, he says he will give that protection “all his political weight”.) Moreover, the real selective war among projects is to come: it will begin in a year or two. “Research has finally become visible as a separate element in the budget”, said a spokesman of the ministry of education, ominously, and in future “it could be re-allocated between the universities”.

To some minds, this re-allocation, favouring some universities for research, is the whole purpose of conditional financing. But this runs hard against the Dutch tradition of equal treatment. A common plea is that the Dutch universities are really much the same: that there is no Harvard or Oxford. The fact is that there are variations, but no common pattern. Take medicine, for example: one university has an 85 per cent success rate in the final examinations; another has under 60 per cent. But, says Professor Veltman of the Technical University of Delft “It’s very difficult to find extreme differences, because from year to year we’ve always made sure we’ve had the same amount of money, the same procedure for attracting professors, and more or less the same

ZWO gives benefit of the doubt

THE Dutch national research council for basic research, ZWO, judged two out of three projects submitted by universities for the protection of “conditional financing” (see below), and rejected one in five. But they claim they were only “marginally involved” in the exercise.

ZWO is familiar with the distribution of grants awarded by peer review. But the council had to adopt a completely different selection procedure, and new committees, to assess the conditional financing projects, says its director Professor R. van Lieshout. ZWO had no role in defining the nature of a proposal; nor how it should be judged; nor even in what fields the council should judge. “In that sense we were only marginally involved”. The minister’s instructions were “too loose, too open” so the ZWO judgements were made “on someone else’s terms of reference. . . Which were not clear and sometimes contradictory. . .”

The minister imposed three conditions, of which the most extraordinary was that the review should *not* look into a project in depth. Rather, as ZWO saw it, the reviewers were only to investigate a group’s record, its potential, and whether its project was serious or window dressing.

But very often all the ZWO committees (specially convened for the purpose) could do was assess records, for the projects themselves were defined so sketchily that assessors could not decide whether they were good or bad. “We could not consider, as usual for a ZWO proposal, who was going to do what when, whether the project was well-planned, whether the researchers had the right contacts abroad, whether they even had the equipment — the review was much lighter. It was more a matter of trust, understanding, and giving the benefit of the doubt”.

Nevertheless says van Lieshout “Very often the people on the special committees knew the groups, what they had done and could do. So they could detect tricks, and fill in what was not on paper. So that’s why here at ZWO” — despite everything — “the conditional financing reviews worked quite well. . .” □

experimental facilities.”

However, Veltman admits occasional differences in quality “because in some places you have a very good man, or a very strong team”, and it is exactly this kind of difference that conditional financing will emphasize.

There is an unofficial target: that in any university, approximately half its research should be covered by conditional financing (that is, have won approval in the national committees), the rest coming unreferred as before. But so far only about 30 per cent of university research has been subject to the scheme, so not enough research has yet

passed through the scheme to start re-allocation. When this happens, in two or three years' time, universities that have gained few approvals will see their un-refereed research funds diminish in proportion, and to those that have more approvals, more funds will be given.

That is when the conditional financing fireworks may begin, not least because of a flaw in the whole system. The refereeing system for conditional financing was established in haste, and it is a rickety, untested Gothic structure, full of anomalies. The best and most obvious projects are getting through safely, but the middling and the unusual face a lottery. But the ministry will keep the system, based on *ad hoc* committees in a complex of different non-governmental institutions, and a set of ambiguous instructions.

According to the ministry, some things may be changed, but not the main structure. However, a spokesman admitted "We have to create more unity from subject to subject. Some judgements were prospective, some retrospective. . .". The ministry has been thinking of improving its guidelines. "There has been a problem of clarity in the decision-making." But "we do not think in practise there were real problems. . . There will be fine tuning."

However the whole exercise was so novel for the Netherlands, it is no surprise that the fine tuning will, in some subjects, be more like a complete change of channel.

In chemical engineering, for example, projects were at least initially assessed by pure chemists with, as one engineer claimed, irrelevant ideas of what chemical engineering research should be. And in biochemistry, a relatively new group in carbohydrates at the University of Amsterdam

. . . there has been a problem of clarity in the decision making . . .

was judged, says a group member, on its publication record by a panel that knew nothing of the work or the group.

The group failed to win approval. But, says the aggrieved group, the panel should have used prospective criteria as the research was building up, and suffered from a "blinker attitude" to the importance of carbohydrates in cell biology. Moreover, the group has set about proving its case by collecting hundreds of letters of support, and independent referees' reports on its work, from around the world.

And beyond such hearsay, the ministry of education, aware of criticisms, commissioned an external report on the assessment procedure. It was prepared by Jack Spaapen of the "Science Dynamics" group of the University of Amsterdam, headed by the English professor Stuart Blume, and details an extraordinarily varied and haphazard process of assessment. Spaapen studied the work of two assessment commissions from the basic research council, ZWO, three international *ad hoc* commissions, and commissions from the Royal



Stuart Blume of Amsterdam's "Science Dynamics" group.

Institute of Engineers (KIVI), the Royal Academy of Sciences (KNAW) and the National Agricultural Research Organization (NRLO).

All adopted "very different" procedures, which went beyond differences appropriate to tackling different subjects, Spaapen concluded. Some used committees to arrive at consensus; some "committees" never met, but interacted only on paper, with decisions taken by the chairman alone; some fed back criticisms to applicants and suggested re-application; some even invited applicants to the review. Writes Spaapen ". . . these problems largely derived from a divergence between the [conditional financing] procedure and usual evaluation roles. Uncertainty as to

the precise function of their evaluation (and as to the consequences of a "no" in a given case) appears to have been common."

Perhaps as a result, rather few proposals were ultimately rejected (about 25 per cent), although the ministry claims that this was more than expected.

Another problem is the fear of increasing governmental control of the otherwise highly independent Dutch universities. The conservative government has not hidden the fact that come re-allocation, it may indicate some priorities in this great swathe of university research over which it previously had almost no knowledge and certainly no control. And since the financing involved is direct, and the assessment procedure so haphazard, since there is no organized buffer, no coherent policy-making research council, between government and spenders, the possibilities for control are great.

To government, the opportunity must be very tempting: but Professor R. van Lieshout, director of ZWO (which controls the 7 per cent of funds normally peer-reviewed) reacts very strongly to this idea: "It is of course a very dangerous development . . . My colleagues in Germany were quite shocked when they heard this. They said 'that's the way it started in the thirties'. They're very sensitive about such things there." In fact, says van Lieshout, there is no serious intention of such political control in the Netherlands, but "sometimes things slip in a certain direction even if no one intends them to; you have to be very careful." □

Universities

Cuts concentrate the mind

UNTIL the mid-1970s, Dutch university researchers were sitting very pretty. The rector of the technical university of Delft, asked if the Dutch universities had been the richest in the world, replied, laughing: "Oh yes!" Buildings, equipment and staff were all available as needed. In Delft, there were two technicians for every researcher, "a figure you will not easily find elsewhere in the world."

Then the oil crisis led to the first budget cuts. At first the cuts were mild. The government (then socialist-dominated) opted for "restrained budget growth" of 1 per cent per year in real terms. In the universities, this became a little harder: a real-terms freeze. But this was just play-acting compared with what was to come.

The new (conservative) government of 1977-78 faced inflation. It sought real cuts — and the university budget shrank by 1 per cent. But these cuts were only in buildings, materials, research equipment, and computers; there were no sackings, and — according to a government spokesman, the cuts posed "no problem". But gradually the knife came closer to the bone. The ministry decided not to compensate

for inflation in 1979. Nor in 1980. Nor in 1981. Then the first real pain: over and above the "inflation penalty", in 1981 the universities faced a real budget cut — in personnel — of Dfl 60 million (around 2 per cent, just over £1 million per university).

Then in 1982, the government demanded further, more sizeable cuts: another 10 per cent. Now the universities were in trouble. The ministry felt that savings could not be found on buildings or materials. The only place for further cuts was staff.

The ministry adopted three main principles:

- First, the cuts had to protect research. So cuts had to be found in education: in reducing the teaching hours per student. (Which, says the ministry, was a high figure in the Netherlands.)

- Second, the cuts were not to be even: said an official involved "we had to identify areas with the least and worst output: we had to make qualitative choices".

- And third, the present (right/centre) government decided not to make these choices itself.

So the problem was thrown to the universities, who had always pretended each

was equal to the other but now had to make distinctions among themselves — or face the minister making them himself. The bluff was called.

Yet it took the universities only 6 months, until March last year, to negotiate the 10 per cent cuts, and indicate where concentration (the elimination of departments at some universities, or their merging at another) could take place. This was fast work. So had there been substantial agreement?

"Yes", claimed one rector "but it led to a lot of trouble within the universities, as the democratic councils did not agree with what the boards had done . . .".

"No", said the ministry, the universities had fudged a lot of issues. Minister Deetman set himself the task of making sense of the universities' proposals, and of reaching decisions which were, his staff claim, "very careful, but on the other hand very clear". After long consultation, in November last year the minister offered each university a contract, covering the cuts they would suffer and a politician's guarantee of defence at that line.

This was it, said the minister: there would be no further changes. Two university councils (the university parliaments) told their boards not to sign: Amsterdam and Delft. But Deetman has threatened that he will simply not pay for departments to be closed under his plan. His list includes 34 departments, from the very small to the very large, which should be closed. Some departments, for instance geology at Leiden, are already closed.

Overall, the cuts aim to save the ministry of education some Dfl 280 million (£70 million) from 1987. But on Deetman's orders, the cuts went a little deeper, reaching about Dfl 310 million to provide a surplus of Dfl 30 million for redistribution on new research projects. Some of the savings will go to biotechnology in Amsterdam; some will be used for a new pharmacological research laboratory in Leiden; some will go on psychology; and some on informatics, mostly in support of a ZWO (national research council) foundation in Amsterdam.

On the whole the government has protected the provincial universities; so the universities of Groningen in the north, and Twente in the east, were "saved"; they also had to economize, but less than the big, central universities in the "ring city" of Rotterdam, Delft, the Hague, Amsterdam and Utrecht where most Dutchmen and women live. As one president put it to this Englishman, "the general policy was that London can look after itself!"

The University of Limburg, for example, will actually get some new departments. "This is a political decision — I think that the academic Netherlands could easily do without that university" complains one "ring city" rector. Limburg is in Maastricht (in the southern tail of the Netherlands), rather distant from other universities, in an old coalmining region,



Although not faced with Belgium's regional troubles (see page 507), the "ring city" of Rotterdam, the Hague, Amsterdam and Delft is looked on with envy by "provincial" towns.

20 km from Aachen in West Germany. "You get the ridiculous situation that the most expensive academic hospital in the world is in Aachen, and the most expensive in the Netherlands is in Maastricht, and you can see the one from the other — it's ridiculous. It's all to do with a decision to redevelop the area" says the complainant.

As for the three "private" religious universities, very little of the religious element is left in them but they have sometimes been able to make use of it during "concentration". It gives them a certain

power, through religious connections, in parliament. For example the Roman Catholic university of Nijmegen was to lose Roman studies, but said this was impossible because it was RC. But the Protestant Free University of Amsterdam had the choice of saving sociology or dentistry, and they chose the latter, and were defended on the choice, whereas on religious grounds the former was more arguable. It thus appears that these universities simply use religion quite cynically, when it is useful to them.

In many ways, then, Deetman has achieved his aim — an uneven distribution of pain, although the unevenness has not always been defensible on academic grounds alone.

But the story of university concentration is not over yet. The next problems to face the minister will come in the courts, where the government must defend seven particular decisions. The most serious — or at least spectacular — concerns the justice of the closure of dentistry in Utrecht, the biggest dentistry department in the country and the biggest of all departments to be closed, saving Dfl 20 million (£5 million). The closure has created much emotion, but the ministry won a first hearing of Utrecht's case in a lower court (in private chambers).

And there are still some other loose ends: in some subjects only the budget cuts have been defined: in sociology, economics, law, and medicine, including the academic hospitals, one of which — in Amsterdam — has been threatened. Deetman will decide in these areas this year if a department or two has to go. □

Degrees on the dole — early retirements

THERE are some 160,000 Dutch men and women with a degree. Of those, 15,000 are unemployed. University lecturers and professors are now adding to the figures, as budget cuts bite deeper.

The Technical University of Delft, for example, has about 4,000 staff, of which 1,400 are scientists. Some 600 people must go — and although the university can choose who should leave, strong university democracy will put greater pressure on the academic than the non-academic staff, the rector believes.

Professors themselves are not exempt, and as a further, nationwide measure, a whole cohort of professors must retire this year. The retirement age for a Dutch professor falls this summer from 70 to 65, to begin a 20 per cent reduction in full professorships in the Netherlands from 2,800 to 2,260 over five years. A total of 150 over-65-year-olds will be leaving in August, most of them not to be replaced.

But when it comes to redundancies, Dutch university staff benefit from being civil servants. The rector of Delft claims he often wonders whether he should take redundancy, because there are so many ad-

vantages. A redundant civil servant may draw 80 per cent of his or her final salary for a long period, and may supplement this up to 100 per cent through a full-time job while part-time earnings (such as consultancies) are ignored.

So the scheme is attractive — at least for well-established academics with good links with industry. But there is a drawback — government has been warned that it might not be able to pay, and that will have to lower the rates, or limit the number of years they pay. So, says the Delft rector, "that makes many people shy of retiring at the moment. . ."

And says van Lieshout, director of ZWO "I don't think it's a very nice prospect at 32 to be told now you can go home with 80 per cent of your salary, and you don't have to do anything anymore. That's not something to look forward to."

But the early redundancies are "a human disaster" according to the president of Leiden. People leaving are often too specialized to find work. "But if I look at the opportunity to cut dead wood . . . I think that in the long term we will have better universities." □

Going shopping for science

IF 1968 brought socialism and democracy to the Dutch universities, the 1980s are certainly bringing reaction. But, remarkably, one "socialist" innovation is surviving: the science shop. Science shops offer a modicum of university facilities (time, expertise, research) to anyone who cares to ask for it.

Typical shoppers used to be trade unions or environmental groups, but the range was never limited. The most common clients (in Amsterdam at least) now appear to be women's groups; the trade unions, while retaining interest in *ad hoc* questions, are pushing the shops towards more programmed, project-oriented work. (There is a project, for example, centred on electronic banking and its effects on employment.)

But science shops were once the leading edge of university radicalism: now they are commonplace. Every university has one, although the first (Amsterdam's) remains the biggest, and they are recognized and supported by the university boards and the ministry.

So how did they take root? More to the point, how was it that the shops' founders — the familiar 1960s rag-bag of left-wing science students and lecturers — did not dissolve into factionalism, as such groups did in almost every other country?

Loet Leyersdorff, one of the prime movers of the Amsterdam science shop, now on the staff of the Science Dynamics unit at the University of Amsterdam, believes there were several reasons, but most interesting among them were two: First, the trade unions gave very early support to the movement, and were keen on practical results. And second, majority rule in the shops' management. There is a strong tendency to compromise in the Netherlands, Leyersdorff explains, and this Dutch tradition is very strong in Amsterdam. "Even old-fashioned Marxists are ready to compromise." □



Posters at the "Science Shop" in Amsterdam.

Research council

Tiny budget — broad spectrum

THE Dutch have one research council, or coordinating body, to cover the whole of basic research — from the humanities to physics. Called ZWO, it is the only national body that allocates grants through thorough peer review (conditional financing aside). This gives ZWO a special prestige in Holland.

But ZWO has only a relatively tiny budget — covering, overall, about an eighth of Dutch university-level fundamental research. According to a recent survey the 13 Dutch universities spend around Dfl 1,650 million (£410 million) on research. The ZWO is Dfl 200 million (£50 million), about half of which is spent in its own independent institutes.

But there is overlap: ZWO institute directors often teach in universities. So there is a passing similarity between ZWO and the French national research council, CNRS. "There's exactly a factor 10 between us", laughs ZWO director Professor R. van Lieshout, a physicist from Amsterdam. "They have 23,000 people, we have 2,300, and there's almost the same factor in budgets. But they have many more institutes than us: for them, CNRS is practically only institutes; here it's 50/50."

But there is a cachet for a Dutchman in being funded by ZWO, as there is for a Frenchman funded by CNRS. "... we like to think so", says van Lieshout. "But it's not true in all fields", he says candidly. "For instance in social science some people think we're not helping enough and are funding the wrong things..."

For historical reasons, ZWO is most prominent in physics. There are five ZWO physics institutes, which consume half the ZWO physics budget; the other half goes to university groups. Of university physics spending, some 30 per cent comes through the ZWO. In some fields — such as plasma physics — there is work only at ZWO institutes.

In biology, the Royal Academy of Sciences (KNAW) plays an important role, mostly in the ecological sciences, but, says van Lieshout, the KNAW institutes are only loosely linked to university life, unlike ZWO institutes.

There are no biological institutes in ZWO, however. The biologists did not want them, it seems. ZWO supports about 20 per cent of all university basic biology, apart from its support for molecular biology through the chemistry division. (There are other substantial sources for biology through the agricultural university of Wageningen, through the medical research foundations.)

ZWO was founded in 1950, and began by integrating physics and mathematics institutes established four years earlier. The main aim was to tidy up the funding of basic sciences. After 1950, ZWO began to create other institutes on the model of the

physics institute (FOM) and the mathematics centre. First to have institutes were chemistry and astronomy; and ultimately ZWO was spawning foundations in subjects such as theology, literature, linguistics and history.

Recently the budget has been "stable" — which in practise, because of falling university budgets, has given it an increasing, although still small, part in university spending. Last year the council won a small real increase (1.5 per cent) "through a technicality".

But next year the budget could go down. The finance minister of the conservative coalition government plans a further large cut in government spending. It is "almost unavoidable" that the cut will hit ZWO, van Lieshout fears "because they've cut the universities already, and protected their research through 'conditional financing'; so it's very difficult for them to go back and squeeze more out. They will be forced to

... in social science some people think we're funding the wrong things ...

cut organizations that haven't been hit yet. We're one of them." If there are cuts, the first item to be affected will be the support of research students, he says.

Nevertheless, ZWO has optimistically requested an increase from Dfl 200 million to Dfl 204 million next year, partly to cover an increase in social science and humanities spending requested by the government; "Whether we can still have an increase for physics, medicine, biology and so on I don't know. We're trying to get one." ZWO has also requested a Dfl 20 million capital equipment budget (on top of the Dfl 200 million).

There should also be some money for ZWO in a recent, major government informatics plan: Dfl 1,300 million (£325 million) mainly on education in informatics from the kindergarten to industry — "The whole population is to suffer from it!"

Has 1.5 per cent been enough to keep ZWO at the top? Probably not, says one ZWO staff member. A few years ago ZWO physicists tried to quantify what increase was needed just to keep up with new experimental techniques: they came to 4–5 per cent a year. But, says van Lieshout "Maybe our equipment budget was a little luxurious in the past here and there but it should not have gone down as far as it has."

ZWO needs to find cash to do something for LEP, for JET, to keep up its effort in astronomy, for apparatus for chemistry, and for a small computer or a computer link for the humanities and social sciences. ZWO is also seeking big equipment for research on surfaces: and would like to do joint research with the multinational

Astronomers spread far and wide

"DUTCHMEN live in a small country and we have to collaborate to get anywhere", says the chairman of the department of astronomy at the University of Leiden, Professor Harry van der Laan. Which may be one of the reasons why Dutch astronomy is so well known, particularly in the United States — where Dutchmen (mostly from Leiden) have headed many key facilities like the Mount Palomar telescope (for a long while the largest in the world), the astronomy departments of Yale, Harvard, and the US Naval Observatory (twice), and a Dutchman (Oort, previously director of Leiden Observatory) was ESO's founding father.

But Holland spends less on astronomy than France, West Germany or Great Britain, whether measured in proportion to either the gross national product or the population. And the country has fewer astronomers per head than the United Kingdom (the Dutch figure is comparable to the French and the German). The reason for the Dutch success, van der Laan suggests, is that Holland has had some great astronomers who have also created great Dutch schools (he mentions Kapteyn, Minnaert, and Oort).

Their students found Holland too small, says van der Laan. So Dutch astronomers colonized the world's telescopes and gained a reputation.

Moreover, in international collaborations Dutch astronomers have insisted that they win their share on the basis of research quality rather than any measure of funding, which given the size of the Netherlands would of course be small. In the col-

laboration with the United Kingdom over La Palma (in the Canary Islands) and Mauna Kea (Hawaii), under which the Netherlands pays a fifth the cost of various telescopes, optical and millimetre-wave, Holland pleaded strongly that it should not



Harry van der Laan — backing collaboration with Britain.

get a block allocation of 20 per cent. "I said we should compete on merit in the single allocation committee — in which of course we will be represented by our scientists" said van der Laan.

According to van der Laan the collaboration with Britain has been developing "beautifully", and has led to a "conver-

gence" of the work of other universities in Holland. The Dutch universities are now all collaborating to build state-of-the-art instruments for La Palma, whereas previously their efforts were much more scattered.

As for ESO, which the Netherlands launched (the United Kingdom does not belong), "it has had a long, slow, start partly because of the remoteness of the place (the telescopes are at La Silla in Chile), partly the trouble of having many European nations working together". But according to van der Laan La Silla is already "a real state-of-the-art observatory, a fantastic observatory". "More and more" it produces the astronomy that it should.

ESO was born in Leiden: Oort called a meeting of the half-dozen most prestigious senior astronomers in Europe, with the idea of collaboration, and the result was the launching of ESO.

Van der Laan would like to extend Dutch cooperation with Britain and ESO. He would like to see Britain and ESO get together, for the later 1990s, to build the next generation telescope. It would be a \$50-100 million telescope, very important as a complement to the Space Telescope "and much cheaper. The total cost of the Space Telescope is now estimated to be \$1,300 million, so the next generation telescope on the ground in 15 million class will cost only one tenth as much and will be astronomically just as important (in combination with the space telescope). "Since we are partners of the UK and members of ESO, we'd be happy to be a catalyst" to such an agreement, said van der Laan. □

laboratories in Holland (such as Philips, see page 501), with which links have been weakening over the past few years.

Ten or twenty years ago it was obvious that there was a healthy job market in Dutch industry, so people in universities were often inclined to do work that was relevant, says van Lieshout. Also the companies toured round the universities looking for talent. "So as I put it there was a lot of *contact* research but not a lot of *contract* research." For the past five years there has been less of a market for academics. There was a period when the universities took a dislike to industry; and now multinationals no longer seek to do all their research in Holland. So the links have weakened "and we must be very careful that they don't get weaker."

Beyond mere finance, ZWO is expecting a re-structuring of its responsibilities and management, in a bill with which ZWO is not entirely happy. But the draft law does give ZWO the right to do applied research, a right it has sought for years. A small, young organization (STW) that now supports applied research in the universities will come under the ZWO wing making one broader-based institution.

Why does ZWO want applied research? "Well first of all no-one organizes that

properly." TNO (the big Dutch national applied research organization) works in its own institutes and not in the universities (except by occasional collaborations). But ZWO, on its foundation, was denied the right to do applied research because of the existence of TNO. "That was a mistake", says van Lieshout.

As for the rest of the ZWO restructuring proposals, all is not well. The government's proposal was submitted to ZWO, the Academic Council, the Royal Academy and the Council for Science Policy for comment. "But the government found the comments rather unexpected . . . strong and rather negative. Since then there has been silence."

The proposal is self-contradictory, claims van Lieshout. First, the government announced it wished to step back and not be directly involved with ZWO, while yet retaining some influence. But in the government's detailed proposals, influence increases. Van Lieshout is happy to accept influence "if there's open dialogue between us; it's OK for a scientist to take up a slightly different line of research that's more interesting to government but just as interesting to science."

Present government influence take the form of an official representative of the

government on the ZWO board. He has right of veto; but he never exercises it. In the proposed reform, the government superficially appears to want less, no more than an observer on the board. Would this not reduce their influence? Said a ZWO man "They want to have their influence without committing themselves in the board of the organization. They believe they have more power as an autonomous partner."

"If you want to make a caricature," says van Lieshout "ZWO will think about science all by itself, and will come up with a proposal; the government will think about it and come up with its own proposal, listening to parliament and to public opinion; and then the two opinions, generated under their own steam, will be confronted and we will come to a consensus."

"Well our idea is that that is not a good way of doing it, and that it's better to meet several times first on a lower level before you come to these views — and only then confront. But the government thinks it better to have the two lines separated and only confront at the end."

And in the detail of the proposal, it turns out that the minister will have to approve "every detail" of the budget. At present, the minister approves only the total sum.

"Of course there was discussion, but there was no official seal of approval on our allocation of the money. Now there will have to be." If the minister disagrees, ZWO may have to make a new budget.

As a further restriction, ZWO would have to consult the minister over any new international agreements. And on international relations ZWO has a rather independent point of view. ZWO is reluctant to enter into generalized international agreements, and it has only three: for exchanges with CNRS (France), CNR (Italy) and the Royal Society (Britain). ZWO has been offered exchange agreements with, for example, the US National Science Foundation, but has decided against them because, says van Lieshout "that kind of agreement we can have anyway . . . we can send people anywhere . . . there's no obstacle. And the universities also can do it on their own. So sometimes we don't even use up all the possibilities under our agreements because people can find their way without us."

However, when ZWO finds there is already international cooperation going on — say among astronomers — then it is willing to set up an international umbrella agreement that will make the collaboration official and "keep the money flowing". That is how ZWO reached an agreement with the British Science and Engineering Research Council (SERC) in astronomy and synchrotron radiation, to share the cost and use of British facilities, "only after it was quite clear that there was something workable, a concrete and very well defined project that would fulfil its aims." Says van Lieshout, "we're very much averse to starting an agreement just to see how it works out."

ZWO has also managed to avoid paying large international subscriptions. So it does not pay directly for membership of the European Molecular Biology Laboratory, although the question of continued membership "comes up all the time" in the Netherlands. Equally for CERN, the European organization for nuclear research in Geneva. ZWO does not pay the CERN subscription either, although "CERN was very much in the balance a year ago in the Netherlands . . . but the minister went there and was convinced".

The European Space Agency (ESA) is also outside the ZWO budget. So is the cost of scientific satellites (such as the infrared astronomical satellite IRAS), which is covered by the ministry of the economy. But there will be no more national Dutch research satellites, the minister said: there is not enough interest in industry, so Dutch space scientists must concentrate on ESA.

And they will do it through ZWO, as the Dutch space research institute, with a DfI 50 million budget for the building of instruments, has just been put under ZWO control. The move is "purely administrative", van Lieshout claims. "But now we have the opportunity — if we want to and after a few years — to shift the balance between space science and other fields of astronomy." □

Science policy

Half a minister better than none

AFTER a ten-year experiment with a science policy minister, the Netherlands now has none. Or perhaps a portion: in the present cabinet, the function has been combined with education in the post of minister of education and science, now held by the scourge of the Dutch universities, Dr Wim Deetman.

It was not a conscious decision to demote science policy — just a way of balancing the books of a delicate coalition of parties. But, says Mr von Spiegel, secretary-general of the directorate for science policy (which used to be the science policy ministry): "In Holland politicians are not interested in science."

Arguably as a result, and while Deetman was preoccupied with streamlining the universities, his captainless science policy

In Holland politicians are not interested in science. . .

director last year lost half its operational budget (originally DfI 70 million, some £17 million), a flexible fund for direct stimulus of research and development, and one of the directorate's most cherished instruments. (Otherwise it merely coordinates budgets under the control of other departments and advises on science policy.)

The loss occurred in a split which gave the directorate's work in "technology policy" to a new office in the ministry of economic affairs (which deals with industry). The new office also took on 10 of the policy staff. Thus von Spiegel and the remainder of his team find themselves rather exposed and isolated. And not only is the directorate preyed upon by the ministry of economic affairs, it is also at odds with the more bureaucratic department of education with which it is now combined. (The education department insists, in particular, on university autonomy, and even in the recent cuts largely allowed the universities to decide for themselves what to cut and what to defend. The science policy department style might have been different, but it was not allowed to play a significant part in the exercise.)

The science policy department is therefore being forced back into a more reflective role: "We will be less busy with operational tasks, with execution, than before", says Tindemans. The directorate will concern itself with the long-term rather than the short. But it retains its powers to coordinate budgets, and the minister of education and science is able to use the department to establish science and technology policies that cut across departmental boundaries — such as information technology, where the department has recently established a major new government action. That is a subject "of very broad importance", says Tindemans, bearing on the economy, education, health, public policy,

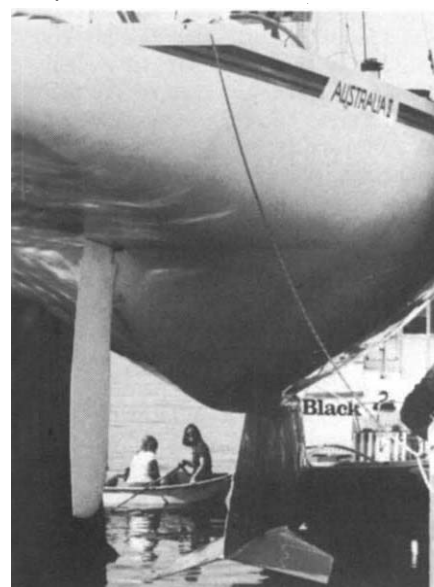
and administration. Hence it made sense to give the definition of an informatics policy to the science policy directorate.

The directorate's DfI 300–500 (£75–125 million) informatics plan makes proposals for the expansion of informatics education (at all levels); offers funds for research, especially in software, and for applications — for example the computerization of Dutch records of physical infrastructure (telephones, cabling, pipes and so on). It also deals with networking and "task differentiation" (somewhat difficult in Holland) between big centres and small ones, involving the introduction of big computers like Cray 1s and Cyber 205s, where Holland has little experience; and deals with the transfer of academic expertise to industry. The acceptance of this report has been a science policy directorate success.

Also, the directorate retains control of the applied research council, TNO.

Intervention on departmental budgets and policy "will take more of our time in the future", Tindemans claims. But before the departments quake in their shoes von Spiegel's view is that such intervention would prove very difficult.

The directorate is also managing an exercise to raise efficiency, and so cut spending, in some 60 government research institutes. Universities are included, but only as a point of reference. "We are looking at staffing and management of the centres" said Tindemans, in order to have an impact on the government budget for 1985. The savings are to come to DfI 130 million (£32 million) or about 5 per cent of the budget, less than the 10 per cent asked of the universities. "We think we can do it", said von Spiegel. □



Dutch technology was behind the revolutionary design for the hull of Australia II, which took the Americas Cup from the United States last year.

Biotechnology

Ambition and a track record

THE Netherlands is planning to make an "explosive impact" on biotechnology within the next couple of years. And they may well do so, not because Dutch companies can lay claim to the world's first genetically engineered vaccine (for pig and cow diarrhoea), and the first monoclonal diagnostic kit (which they can) but rather because the whole of biotechnical Holland is now, as one biotechnologist puts it, "in the start position".

The keys are context, and scale. First, Dutch biotechnology is forming within a coherent, flexible, apolitical, imaginative and believable national "strategy" (to call it a plan would make it sound too centralized and deterministic), in which basic research has a firm place. Second, although in a larger country such scheming can fail through excessive scale and, in particular, poor communication with industry, Holland is small enough that it really has a good chance of creating "The Netherlands Ltd". (History and geography have made cooperation natural to the Dutch.)

The strategy is the effort of a small group of biologists and industrialists, the national advisory committee on biotechnology, which has been giving direct advice to the Dutch cabinet. Its chairman, the most

than technologists, but there are moves to change this. The idea, Schilperoort explains, is to orientate university and government laboratory research "a little bit more to the market." University research may be as fundamental as scientists wish but "It shouldn't conflict with research in industry . . . we want to make it a source of results and people."

The committee does not want to create a biotechnology centre, believing that such places grow "sleepy". "Instead we make centres on certain themes, which have to compete all the time for funds." Thus the committee has somewhat by-passed the national applied research organization, TNO, which might on the face of it have been the focus of the biotechnology programme.

"TNO got a substantial part of our money, we have stimulated their programme in biotechnology, but they will have to do better if they want to get more . . . It's grown too big . . . it's surprising . . . TNO was thought to be more commercially oriented than the universities . . .".

On international collaboration in biotechnology, the committee has an unusual, Europeanist attitude. "We like to collaborate in areas in which we are strong. So we've chosen three areas for collaboration: agriculture, waste treatment and pollution, and process technology. It doesn't make sense to collaborate from weakness. This is nationalistic. Collaborating from strength then we have a chance to be a strong continent. If we only act at weak points we will never be strong!" This, says Schilperoort, "is our signal to Europe". Bilaterally, the Netherlands is making plans with Switzerland and Germany, and is in discussion with France and the United Kingdom.

But back home, how far will this programme protect basic biology in the Netherlands against university cuts? "We are quite successful . . . there are really severe cuts, but from the money we have now we can support a lot of research." The committee itself has Dfl 80 million to spend this year, and there is another Dfl 30 million to be granted through a special priority-area committee. "We are making corrections to the cuts in the area of biotechnology."

And Schilperoort, who has had a strong personal influence on the development of the Dutch biotechnology programme, believes in basic research: "Our basic philosophy is that we need excellent, well-organized basic research — otherwise you are out . . . It's very important to stimulate the creativity and originality of fundamental scientists, because you can't predict what they will find; we should only say, why not try working on organisms a little bit different from *E. coli* and see how far you can go . . .".

As for posts — "that's very important" — the committee would like to create university positions, in collaboration with the ministry of education; but only in the areas that will underpin industry's priorities.

There should be no secrecy in the universities, the committee believes. Fundamental research should be open research, says Schilperoort, both on principle and in practise. "You can't oblige a member of a university to sign a secrecy agreement . . . who will pay the money if he is sued? . . . from a practical point of view a university can't keep secrets."

But this is where the government applied research centres, like TNO, can come in. There, there are no problems of secrecy. So industry could transfer a successful, basic



Chairman of the national committee, R.A. Schilperoort.

university project to the applied centres for development. "And we are thinking about how to set up small companies, seeing what mistakes we can avoid . . .". In the small entrepreneurial companies established abroad, market research has often been weak, they have begun without clients, and many companies will just fade away.

The Dutch programme is coming after the first wave of biotechnical excitement, but this very fact may give it an edge. There are many markets left, Schilperoort believes. And it is the large companies which will lead the way. And they are prepared, having taken the biggest, most expensive scale-up steps — "much more expensive than a small amount of research". For example, Shell and Gist Brocades have created a joint, Dfl 300 million, biotechnology laboratory.

Such things, plus good links with the universities, will create the Netherlands biotechnology enterprise. "Our Dutch industry is prepared . . . They have good projects already and there needs to be a good, continuous, realistic flow . . .". And that, the committee is creating. □

From a practical point of view a university can't keep secrets . . .

unacademic of academics Professor R.A. Schilperoort, plant geneticist of the University of Leiden, says the committee will wind up in May 1985, its work complete. The first real marketable products will emerge from the committee's Netherlands Ltd, through the big companies, within one or two years, Schilperoort believes. One of the major product areas will be in waste-water treatment, he expects.

The committee has asked the big Dutch industries in the area (including Shell, Gist Brocades and Unilever) what research they would like to be done in Holland, and who they would like to do it. "All the companies are showing great interest. It's very delicate — companies like to be very confidential." But through individual, one-to-one conversations largely with Schilperoort's aide, who has signed a secrecy agreement, the advisory committee has got to know, at least in outline, all the Dutch companies' plans in biotechnology. This makes sensible strategic planning on a national scale a reality — whereas in France, for example, similar moves by the socialist government were met largely by non-cooperation from industry.

One problem has been the predominance of projects initiated by scientists rather

Philips

Too big for a cautious Europe

By far the largest research organization in the Netherlands is not public but private: the 2,000-strong Eindhoven laboratory of Philips, the electrical equipment multinational. And beyond Eindhoven, Philips, the maker of the technically brilliant but in marketing terms unsuccessful Laservision, the silver disk, also employs a further 2,000 researchers overseas in England, France, Germany, Belgium and the United States.

Philips' research budget of Dfl 500 million (£125 million) is more than double that of the Dutch national research council (ZWO). Its development budget is Dfl 1,500 million (£750 million), supporting a product range that began with light bulbs and now covers almost everything electrical outside power engineering. The company is also the biggest employer in the Netherlands. Some 22 per cent of its employees are Dutch — but only 7 per cent of its sales. Eleven of its 12 divisions are managed from Holland, but its directors see the company as primarily European, with a quarter of its sales, half the factories, and two-thirds of its employees in Europe.

Philips' greatest single research strength is in materials, claims Philips vice-president for research Dr A.E. Pannenberg. The original lamp research was the investigation of materials under extreme conditions. From there Philips invented ferrites, had a flying start in transistors, works now in ceramics, metals, glass, fluorescent materials, semiconductors. In fact, breadth is one of Philips' greatest strengths — the development of Laservision embraced lithography, precision mechanics, optical technology, servotechnology, plastics and more. But yet, Philips is broadening still, by setting up a joint venture with the American communications giant AT&T on digital switching, aiming first at the European market. The objective: economies of scale in the development of equipment.

In telecommunications Philips' markets were not big enough. Inevitably, perhaps, the company has done well in the Netherlands, "and we've filled Saudi Arabia to the point they've got telephones on every palm tree", says Pannenberg. Philips also has a market in the old Dutch colony of Indonesia and a modest position in Peru. "But if you add up our markets, we can't bear the costs. We were very happy that AT&T was available. We are complementary." AT&T has marketing in the United States, and Philips has competence in the worldwide CCITT standards, which the enormous American market has previously been able to ignore.

Cooperation with other companies in Europe, however, is developing only slowly. But the European Commission exercise on coordinating European research on information technology, Esprit, was a big step forward.

On academic cooperation, Philips' re-

search works almost independently of the Dutch universities — except for staff, which are recruited young, spend 5–10 years in the research laboratories and then move into the product divisions. The average age in the Eindhoven laboratory is rising a little, but there is a firm policy of moving people on, in order to be able to recruit up to 200 graduates a year.

Nevertheless, Philips does not go in for contract research with the universities, or other outside laboratories in Holland. There were more links before the university "revolution" of 1968–69 but then the company pulled back — as did the universities

... most of our competitors have died in the struggle. ...

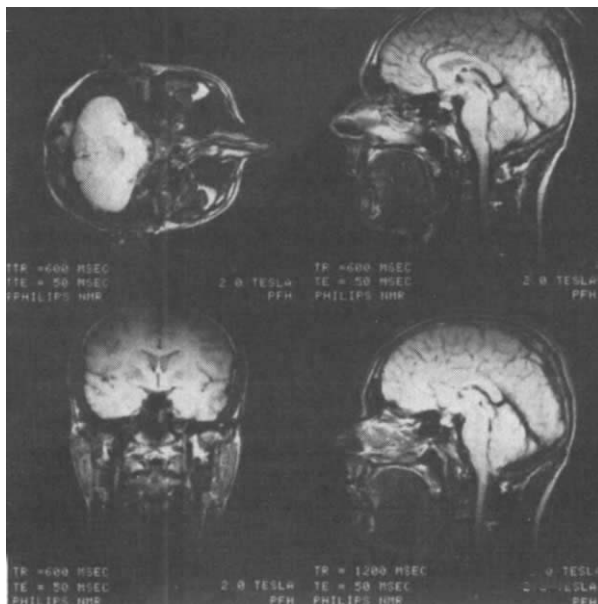
at horror in involvement in matters commercial — and since then the two sides have been drifting further apart. There is now a new climate of willingness in the universities to cooperate, but, says Pannenberg, "The quality of university science is fair but not particularly good in this country." The "egalitarian wave" of 1968–69 led to a reluctance to develop centres of excellence. So there are very few places in Holland with which Philips would really like to cooperate. On the other hand, in computer aided design Philips has "intense and effective cooperation" with the Flemish university of Louvain over the border in Belgium.

But despite its arguably world-class research effort, which to some extent justifies its disdain of Dutch university research, Philips profits remain, to quote vice-president Pannenberg, "unacceptably low". The problem, he says, is less with Philips than with the conservative, unadventurous European customers. If Philips in Europe is healthy, says Pannenberg, then Philips is healthy worldwide.

But in Europe "we lack the leading-edge customer. It makes life damned difficult!" Thus the true strength of America's Silicon Valley, claims Pannenberg, is not that there are so many highly competent silicon foundries, but that these foundries "are embedded in a place where you have the leading computer manufacturers, the leading weapons systems people, the leading automation people of the world who suck the products out of these foundries". By contrast the weakness of Europe is the cautious, reticent, non-innovative, wait-and-see attitude of the customer, whether institutional or individual. Nevertheless "if you look around Europe, we've done quite well. In fact most of our competitors have died in the struggle."

However, according to some outside observers Philips also suffers from a number of structural problems, the first being the separation between the research and product divisions which (say the critics) has allowed research to get carried away with its own ideas. Philips claims to have cured that problem recently by introducing more interaction. And some in Philips will admit that, perhaps, the quality of Philips' marketing does not match up with the quality of its research. Philips could do with a strengthening of "strategic marketing" — the kind of thing that might have taken into account at an early stage that the consumer was not going to be too interested in a video disk (Laservision) that could play but not record, however beautiful the technology, when it came on the market long after recordable videotape, and at a higher price. Philips is now working on home recording onto the disks, but there is a problem of stability of the record, and marketing tactics are now having to shift to the much smaller "professional" market. But perhaps, with a little more awareness of real markets, and a little less complaining about the backwardness of European consumers, Philips still has a bright future. □

THESE images of an adult head are another product of the Philips research effort. From the laboratories in Hamburg, West Germany, they represent the first 2-tesla images of the human head by nuclear magnetic resonance imaging.



Belgium

Universities poor and divided

UNIVERSITIES everywhere are struggling for money and posts. But the six full Belgian universities can claim a certain notoriety — as an extreme case.

The chief administrator of the national research council (FNRS) claimed recently that Dutch researchers have twice as much money for research as Belgian, and the French four times as much, and although “money for research” was left undefined, figures do show, for example, that Belgian university research funds, per undergraduate student taught, are less than half those in the Netherlands (see Table 4, page 493).

The best basic research survives, much of it only by shifting emphasis heavily towards the applied sciences. The national government has been supporting applied science with fashionable zeal — although some responsibility for applied science has been transferred to regional governments established in 1980. (One of these, Flanders, is taking up the task with particular excitement, with Wallonia a close second.) But in ten years there will be no new science in Belgium to apply, say many voices, as the basic sciences are more and more neglected . . .

Another course for certain lucky groups is to hang on to an unreliable lifeline thrown by the late prime minister Théo LeFèvre back in 1970. This is a prime-ministerial fund for “excellence”, the “concerted action” programme (the “concert” is with the government). The programme, as conceived by LeFèvre, offered temporary relief to important groups whose costs were rising too fast for the standard funding mechanism to cope. (Their budgets — even those of FNRS — are keyed to student numbers.) But temporary has become permanent, and many important groups now rely on concerted action funds to do their basic work; while, nevertheless, the continuance and allocation of the funds is unpredictably tied to government policy.

Yet despite the troubles, Belgian science is strong: molecular biology, in particular, has a worldwide reputation, and can boast two Nobel Prize winners since the Second World War (of which Christian de Duve, the discoverer of lysosomes, is still active with his international Institute of Cellular Pathology in Brussels). Physicist Ilya Prigogine is another renowned and energetic Belgian Nobel Prize winner; he also works in Brussels.

But there must be a question as to whether such success could ever recur in present conditions. The big slide began in 1976, when the Belgian Government began to seek savings on university spending. At the University of Ghent, for example, 20 years ago salaries accounted for only 70 per cent of income, with the remainder used for research. (This was the so-called “first

stream” of research support, distributed within the university by a local committee as in Holland.) But now some 90 per cent of the budget goes in salaries; so the flow in the first stream has slowed by a factor of three, to a mere trickle. Said Professor Jean Brachet, biologist at the Free University of Brussels, “I was once on a committee here distributing money for apparatus. But now I’m told it’s almost useless to go to the meeting because there’s so little money to distribute.”

As for posts, the universities face, since last year, a demand to make 10 per cent cuts in academic staff over the next seven years. According to Ghent plant geneticist Professor Marc van Montagu, in practice his faculty has made no permanent appointments at all for the past two years. (The same is true at the Catholic University of Louvain-la-Neuve, according to the dean of science there.) Says van Montagu: “Now we have to handle the university like a high school: if we’re short of a biologist to teach a course we have to ask a chemistry teacher to do it: we’re reaching that level. . . The problem of jobs is hitting professors as much as assistants.”

And to add to their relative poverty, the universities suffer a special Belgian pain. Like all Belgian institutions, they face increasing rifts and tensions between the Dutch-speaking north (Flanders) and the

French-speaking south (Wallonia). Four of the universities even make bad worse, as they suffer from a further historic division between Catholic and non-Catholic. As a result Belgian academic funding is all but divided into four equal pieces. Certainly it is divided in two — Flemish and Walloon — thus hindering the selective distribution of meagre resources. An exercise such as in the Netherlands, where the universities were “concentrated”, departments closed, and people sacked — according to what were at least nominally judgements of quality — would be inconceivable in Belgium, as it would be far too inflammatory in regional or denominational terms.

Further, while the mobility of academic staff throughout Europe is bad, mobility in Belgium is nil. A budding researcher who arrives at a university at 18 is likely to stay there for life — if he or she is lucky enough to get a post.

But to take a somewhat Machiavellian view, there is one good side to all this: the near-anarchy has allowed certain pragmatic researcher-princes to establish their city-states. Just as princes raise taxes wherever they have influence, so do these researcher-princes raise funds; and the luckiest, most pragmatic and most persuasive may thus have been able to accumulate a greater relative income than they would have done if policy were more centrally controlled. Thus there is a greater presence in Belgium of centres of excellence than there is in neighbouring democratic Holland. □

Actions concertées — lifeline of the strong

IN the 1960s Belgian basic research was funded well enough through the university budget with grants from the national research council (FNRS). But towards the end of the decade molecular biology, and some other sciences, outgrew these funds — and Prime Minister Théo LeFèvre started a fund, dubbed “actions concertées” or “concerted actions”.

The grants, beginning in 1970, were substantial and highly selective: the aim was to support excellence and only the strongest groups received them. They lasted five years at a time and could reach BF 10 million a year (£125,000), compared with university funds which were rarely more than a tenth of that for supplies. One microelectronics laboratory — at the Catholic University of Leuven — increased its size tenfold in this period.

But will they last? Staff at a key laboratory in molecular biology, at Rhode St Genèse near Brussels, founded by Professor Jean Brachet, have their fears. Brachet has retired, and now the contracts are decreasing; “and we are told that in two years they will finish”. According to Brachet himself “the action concertée has not been the main source of funding for our applied projects, but in a field like embryology, when the action concertée is finished

it’s the end.”

However, Brachet is not too perturbed: the science policy programming office of the prime minister that distributes the grants “say they want new projects — but I’ve been involved before in helping distribute the money and while they say every time it’s the last time, they continue. There



Jean Brachet — optimistic despite budgetary pressures

are not so many groups of this size and quality in the country.”

Incidentally, Brachet also met the famous LeFèvre, who many in Belgium regard as having saved basic science. “He realized”, says Brachet, “that to get people into research of any kind was an investment for the country, even if in the long run he was interested in boosting applied research. □

Industrial research council

Basic PhDs recipe for success

IRSIA, the Belgian national council for the stimulation of research in industry and agriculture, is a good deal more than it might seem from its title. Contrary to expectation — given Belgium's current political attachment to applied research — IRSIA plays an important role in maintaining the fundamental sciences.

Universities depend on IRSIA for research studentships, even in the most basic sciences. (IRSIA merely limits the field to "science", in the English sense of the word.) The council provides 600 such grants a year, awarded on the basis of interviews, compared with the mere 100 from the national (basic) research council, FNRS, which are offered to those with the best marks in first-degree examinations, and cover all academic disciplines.

IRSIA conducts 900 interviews for the studentships at its headquarters in Brussels every year. Apart from university assistantships, which are few, IRSIA claims to cover 80–90 per cent of students working seriously for a PhD.

But why should an applied research council take on such a job? It seems the object — which dates back to the council's post-war foundation — is to provide research-trained scientific manpower for industry. And IRSIA's view is that 'research' requires a certain state of mind, a certain approach, that must be learned before a student considers application.

It is possible to do a PhD in Belgium with grants paid neither by IRSIA nor by FNRS, but by industry. But, says IRSIA's director Dr J. van Keymeulen surprisingly: "this is not the best way to do it: then you have to produce some applied results . . . but a PhD is something else . . . an education".

All IRSIA asks of a professor proposing a candidate for an IRSIA studentship is that he or she must guarantee that the subject will lead to a doctorate. IRSIA claims to take no control over the subject matter of the PhDs, although this occasionally requires a little heart-searching. For example "we asked ourselves several times what the students of Professor Prigogine [Nobel Prize winner in irreversible thermodynamics] would do after that training in theoretical physics. But none of his ex-students is unemployed! So their training was very good because their scientific environment was good — not only Professor Prigogine but his co-workers also. I don't say the subject is of no interest — but it is important on the second level. The environment in which you live — that gives you your training."

Nevertheless, the system might not have survived if the studentships had not also acted as IRSIA's antennae in the university system. "They give us a very good idea what is going on in the university laboratories". They account for only 10 per cent of IRSIA's turnover; but the rest con-

sists of various forms of aid for industrial and agricultural research, much of which finds its way back through joint projects into the university system. There, the studentships help IRSIA management keep in touch with who's who and what are present research trends.

But is all this not just rationalization of a historical circumstance, that the university PhD system began to depend on IRSIA grants? Is it truly a policy? "That takes us back to 1946", explains van Keymeulen "when IRSIA was created out of the

FNRS. FNRS's job was mainly to sustain professors. . . We aimed to sustain projects in industry and agriculture. And we gave scholarships. (We started with 10.) We needed the manpower: Belgium was really underdeveloped in research and development. All we had was universities. The IRSIA idea was to create something in industry — and we needed the people." This historic role has become bigger and bigger.

But was it never the role of FNRS? "No. They selected the very best people. . . the candidates for Nobel Prizes. We had another group. We said we wanted good people, who could give good service to industry. That was our role." □

New studentships plus job in industry

THEY are being dubbed the "Maystadt boys" — although it is an equal opportunity offer. Science and budget minister Philippe Maystadt has established a budget of BF 900 million (£11 million) intended for younger students, just out of university (or having just finished a PhD), to work in fundamental research and learn techniques that would or could lead to industrial application.

Each grant lasts a maximum of three years. But if the person is hired by industry within that period, the laboratory has the right to hire someone new, again for three years. If the person is not hired by industry, they lose that position.

These are not postdoctorates — nor PhDs, although it is not excluded that a doctorate could be earned — but research training exercises lasting 1–3 years. Students on the biotechnical part of the programme, for example, have to learn the techniques of the kinetics of immobilized enzymes, or cloning or other techniques.

"It is a very intelligent programme", said one laboratory director just after a

telephone call telling him he will have five of the posts to distribute. "It's really useful, as you will be able to get on with your own work on the condition that you give training to someone in basic techniques." And Maystadt's project, in fact, covers a lot more than biotechnology — a whole range of scientifically and technologically interesting fields.

However, not everyone is so pleased with the idea. The students must be young — they must never have had a job before. "And", said another recipient "in every contract we get nowadays it says that any result that derives directly or indirectly from the work will belong to the Belgian state. These people get BF 100,000 a year, about a third of what they need to work in genetic engineering, just for materials, so you have to find another two thirds. But each source of money says that everything that will be done belongs to me. . . And in some cases in these grants they forbid publication."

"But — these are 200 positions, and that must be basically good. . ." □

Research opportunities

No room for manoeuvre

It is still possible to do a PhD in Belgium, although the number of grants is falling. But at the postdoctoral level? The constant refrain is that there are almost no postdocs in Belgium.

Universities can create permanent assistantships, but a law of 1976 restricted the number to below 40 per cent of the total number of assistants; and most universities have already exceeded that figure. "We have had no new posts at Louvain-la-Neuve for two years — at any level, in any faculty", according to Louvain-la-Neuve's dean of science, Pierre Macq.

At the same time, fewer students are interested in staying on for a PhD. For example, in 1983 a dozen new graduates from Louvain-la-Neuve sought research studentships from the national research council, FNRS; but in 1984 there are only four applicants. "It's a general situation:

people don't believe in the future. The funds are going down but the demand is going down also", says Macq.

In Belgium, there is nothing like the French CNRS, or the British research councils, to provide postdoctoral posts outside the university system. FNRS has no laboratories. It has a few coveted permanent posts, but these — intended to be stop-gaps but already 12 years old — are almost vanishingly few and offer no career structure. To compare with the French CNRS, there is no level of 'director of research' which 'makes life uneasy' for an ambitious young scientist", says Brachet.

Even so the competition is "incredible" for these positions. Says Professor Nicolas Glansdorff, a Brussels microbiologist "It has become almost impossible for a good — let's not say excellent — researcher to get one of these posts. If you look at the curri-

culum vitae of the winners you would say that most of them should be professors — and more than that for some of them. The problem is that in Belgium where the scientific level is quite good, we have each year maybe 15 people who've published in *Nature* and *Cell* and *PNAS* competing for one post! It's completely crazy."

So the unlucky ones in Glansdorff's laboratory — some of them in their 40s — live year by year, go on unemployment benefit for a time, come back again — "it's really traumatic for them".

At FNRS, these life fellowships are described as "the *nec plus ultra*". Yet they were introduced only as a 'parking lot' when university posts began to tighten up in the early 1970s. It was intended that universities would absorb them when the situation eased.

But the situation never did ease. "Now we have a completely new and very difficult situation", says Brussels engineering professor André Jaumotte, who was FNRS president when the posts were created. "FNRS has reached the maximum number of posts it can fill, limited by its budget. This year, in the University of Brussels we have no more than four posts of our own to fill; at FNRS there are three posts on the French side in all subjects. We are blocked at FNRS . . . at university . . . and we have the economic crisis."

"It's very very difficult now to get a new man into research. . .", says Jaumotte.

The FNRS scheme began with ten Flemish and ten French posts, in all academic fields. Later it declined to seven

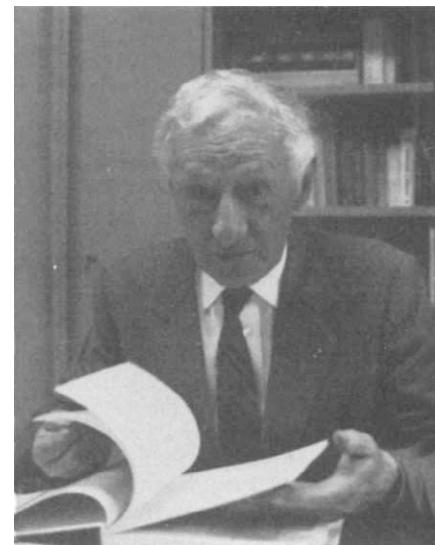
Flemish and six French, because Jaumotte had imposed the sensible rule that if a university failed to give the fellowship holders a job, then they could not get any more such posts. FNRS had relied on the universities taking them up. It was not supposed to be a career position — but it has become one. In 1984 FNRS will have six Flemish and three French posts to offer

Fifteen people who've published in *Nature*, *Cell* and *PNAS* competing for one post . . . it's crazy

— plus another two or three on the Flemish side where some universities are expected to give some existing holders posts. The uneven division between the regions is a consequence of the fact that the Flemish universities are younger and still growing.

Inevitably, with so few posts to offer, particularly on the French side, intrigue begins to play an important role in decision-making. Says Brachet "When the last FNRS director died, they didn't fill his post. So the council is now headed year after year by the rectors of the universities. There should be more independence, because finally it's the rector in charge at the time who decides."

"For example when we try to get a researcher a permanent post, we do what we can in the committees, there are fights and so on, but there are more than 20 committees, one for each field, each proposing candidates — and finally the rector in charge makes the selection in an arbi-



André Jaumotte, the man who introduced the "parking lot" posts.

trary way. So you know that if your man has been classed first, the great thing is to work on the rector, and convince him that your man is the most excellent!"

To put rough figures on it, FNRS has a five-to-one surplus of demand over supply for research studentships; and for permanent positions ten to one. The Flemish-side administrator of FNRS considers that FNRS could double the number of research studentships without any loss in quality. "For the permanent jobs it's awful. We could take 80 per cent of the applicants."

And without posts in Belgium, some of the country's best young scientists are leaving — particularly from the French region to France, where posts and funds are much easier to come by. Said one Walloon group leader: "Two of my students are now *chef de service* at the Institute Pasteur in Paris, one is a director of the French genetic engineering company Transgène, others are in Lille, Montpellier . . . There really is a brain drain."

Even some senior people are leaving — like plant geneticist Professor Jeff Schell who was nominated director of a Max Planck institute in Cologne and now comes in to his Ghent laboratory one day a week — "this is a part-time brain drain!" said a colleague.

Another example of the problem is a researcher who as a student took a joint best first class degree in the Free University of Brussels. He was lucky enough to win a national prize for his thesis, which got him a permanent FNRS fellowship. He has been a year at the Massachusetts Institute of Technology, two years at Yale, and then came back. He might have expected a professorship — but the place he might have taken at the university, shortly to be vacated by a retiring professor, is to be abolished to save money. Anyway he would face the poor funding of science in Belgium. Now he has been asked by the French Institut Pasteur (Lille) to work in France. He would be able to take with him

In or out — a "scandal" over EMBO

BELGIUM's position in the European Molecular Biology Organization (EMBO) and the laboratory (EMBL, in Heidelberg), is somewhat confused. The story begins with the Brussels biologist Professor Jean Brachet. A few years ago he was seeking to set up a national or international biological laboratory in Belgium, on behalf of Belgian biologists. He met resistance at every level, and "that's why I was among the first to think about EMBO". Brachet played an important role in the establishment of the organization. But amazingly, Belgium is still not an official member. And Belgium does not contribute to EMBL.

"What happened", says Brachet "was that after pressure from our Nobellists Claude and De Dève the government admitted that EMBO would be a good thing, but they said that since EMBO planned to have a laboratory, and Belgium did not want a part in it, they could not join". But they pay indirectly: they give the EMBO subscription to FNRS, which pays, but the Belgian state has no official place in the organization.

This leads to a diplomatic anomaly. There should be an official representative of every EMBO state on the EMBO

council, but because Belgium does not have official membership, it has no such rights. Belgium sends the general secretary of FNRS, who is allowed to listen, but not to speak — the government, it seems, fears that becoming full members would mean coughing up for EMBL.

And despite the original reluctance to establish the laboratory, Belgium *does* use EMBL. "But we are really ashamed — we do not feel comfortable", says Brachet's successor Professor Renée Thomas. "EMBO treats us very generously, in the number of people from Belgium who are elected and so on — chosen on scientific grounds alone. We have young scientists going to EMBL and they treat us as if we gave the money. It's scandalous."

EMBO costs some BF 1–2 million, and it would be another BF 25 million for EMBL. "Greece pays; Finland pays; we don't. I asked Mme Dehaux, the director of the government science policy programming office: she said listen, "we haven't even contributed to CERN (the European nuclear physics research laboratory) for two years; why do you want us to contribute to EMBL?"

The CERN payments have now been made; EMBL is still waiting. □

three people for three years, with a permanent post for himself. "It shows people have been well-trained here, at least . . .", commented a colleague.

However, a senior official of FNRS, has a more sanguine view. "It's a very tough problem" he admits. "On a first look, you would say yes, there is a brain drain, in absolute numbers. But is the final output a

brain drain? I know a lot of people who emigrated to the United Kingdom or the United States, who have professorships there, who are training Belgian students who come back and enter industry here — for example in solid state, bioengineering, molecular biology. There is a brain drain, but the effects may be positive in the long term." □

Research council

Strength through division?

THE Belgian national research council for basic research, FNRS, is divided in two, almost literally from top to bottom. Downstairs in its tall Brussels block sit two women, in identical uniforms, side-by-side in identical glass boxes. Above them are different signs: one reads "reception" in French, the other in Flemish. A visitor must be sure to speak to the right receptionist: the first will connect you only to the French side of the council, the second only to the Flemish. Money is divided just as automatically: according to a politically agree formula (called a key — Belgium has many keys), some 54 per cent of FNRS funds go to the Flemish side and 46 per cent to the French.

Crazy? No, says an ex-president of FNRS, engineer Professor André Jaumotte (who is also ex-rector of the French-side Free University of Brussels), this *a priori* division keeps political, regional conflict out of scientific discussions: given the key, "there is no possible argument about the budget, so you can discuss quality without complications." The system results in "some duplications . . . it's not perfect, but it's not so catastrophic".

The Flemish-side administrator of FNRS also backs the division: because it provides independent referees. "Our boards of referees are composed of ten scientists, five French, five Flemish. They advise as one body, but in two budgets. So, say, in discussion of a Flemish project, there are five French referees who are entirely independent; and vice versa.

In fact, says the administrator optimistically, "regional divisions are not worsening the problems of Belgium — they help find the solution! I'm quite sure of that . . .". Belgium, geographically, is the south of the north and the north of the south. So, "two mentalities meet here . . . Here, in this very small country, Anglo-Saxon and Latin attitudes stimulate each other.

However, according to a Walloon biologist, the cultural division of FNRS spreads cash too thinly. "Instead of backing a good group in one subject on one side of the country, and a good group in something else on the other, everyone is trying to do the same thing — every sum is divided." This can mean constructive competition, though says the Flemish FNRS director.

The FNRS formula of 54 per cent to Flanders and 46 per cent to Wallonia is not fixed. It is based on the numbers of Belgian graduate students in the two regions, and it shifts as those numbers shift. The trend favours Flanders. In the 18–30 age range, 65 per cent are Flemish speakers and 45 per cent French, and university attendance is increasing in Flanders, whereas it has stabilized in Wallonia. So the balance is shifting further and further towards the Flemish. It will eventually stabilize at

about 60/40, the Flemish administrator believes.

But these ratios are very new. A decade ago, before the key, the French side used to dominate. "For a long while all scientists spoke French, even in Flanders, and there were few Flemish students until after the Second World War", claims particle physicist Professor Pierre Macq, dean of science at the (Walloon) university of Louvain-la-Neuve. "So the Flemish demand for grants from FNRS was low; for example even in 1970 Flanders had only 30 per cent of all physics research in Belgium, although the population was 60 per cent Flemish."

But, Macq admits "it really was an anomaly". So, at the beginning of the 1970s, it was reasonable that the Belgian premier Théo LeFèvre should determine to give new funds to Flemish fundamental research, without decreasing the French side, until the Flemish and the French sides were balanced. Only half the plan worked. The government established the key, which would give Flanders its fair share. But then

FNRS — cream but no cash

THE Belgian national research council (FNRS) has always aimed to provide for the cream of Belgian scientists, mostly through peer-reviewed grants and — for the past decade — through posts to be taken up in universities. It remains the best institution to which Belgian fundamental scientists can turn for peer-reviewed funds. But FNRS simply has too little money.

And FNRS funding has declined. The budget of FNRS and its "associated funds" for medical research (FRSM), and inter-university and interdisciplinary research (FRSC) fell by 5 per cent in real terms between 1979 and 1983. Over the same period the nuclear and particle physics budget (another associated fund dubbed IISN) fell 16 per cent (excluding international subscriptions).

Contrast this with the applied research council IRSIA, which enjoyed a real-terms rise of 20 per cent, and the whole state budget which rose 37 per cent!

So why has FNRS been neglected? In these political times in Belgium, one fact stands out. Since FNRS focuses on "quality" and peer-review, politicians have no power to control its actions. Political power over other sources — such as the prime-ministerial funds (the *actions concertées*), or national programmes (such as one on energy research) can be much greater and so FNRS is forgotten. That may be the real reason why the FNRS budget has been declining steadily.

Apart from posts, FNRS also gives small research grants to individual researchers — nominally from BF 50,000 (£625) to BF 1,000,000 (£12,500) for 1 to 3 years. The very maximum is BF 1 million for one year but such sums are rare. According to Brussels biologists the sum of BF 500,000

(£6,250) for a year would be "a big grant at FNRS. . . We got BF 50,000 for three years to work on the properties of a small parvovirus . . . which is very little money . . . with no personnel".

The budget for FNRS, excluding its associated funds, is BF 858 million (£11 million) in 1984. Nearly 85 per cent of this goes to studentships and fellowships. The associated foundations have around BF 2,000 million (£25 million) to play with. The funds of FNRS proper are tied to the university budget. By the 1971 university financing law, FNRS receives a precise 4.44 per cent of the running costs (excluding salaries) of the six complete universities. "I've no definite idea what the comparable figure would be in other countries" said an FNRS official "but it must be two or three times higher."

If FNRS were lucky enough to win a budget increase, how would it spend it? "Mostly on research students" said a Flemish-side official "to increase the quality and number of PhDs, for industry as well as university life. Second, to research contracts, to stimulate inter-university and interdisciplinary work." (The Belgian universities are very monolithic, and this would help to break the barriers.) "If the R in R&D has no basic component, then forget your applied, your development. We have to go back to basic research. This is not just special pleading. You must do the research, and train people — the PhDs — who will be able to go out to industry and come back to pick up these ideas."

"We really need double the money we have now. Otherwise I fear for the future — not just for the universities, but for the people of Belgium . . .". □

came a budget crisis; and the FNRS budget was frozen.

There was no new money to pay for the new Flemish share; so since the key had been agreed, the government could do nothing but decrease the French funding to pay for the Flemish increase. So while all Belgian scientists have had to face constant or decreasing funding since 1977, in the French part the problem has been much worse, Macq claims. "The objective was admirable" says Macq "but the result in Wallonia was disaster".

Take fundamental nuclear and particle physics, for example. They are funded through the IISN research fund (an associated fund of FNRS). IISN funding of research in Wallonia fell 20 per cent in real terms between 1977 and 1983, says Macq,

whereas Flanders saw a 24 per cent increase. In research council funds other than IISN, there was a net real decrease of 11.5 per cent on the French side from 1977 to 1982, but an increase of 1 per cent on the Flemish, according to Macq's calculations.

Macq also criticizes trends in the national fund for applied research, IRSIA. "The main goal of IRSIA in 1950 was to expand research fellowships in Belgium, because the number of PhDs was very low before and after the war, except in chemistry. But between 1977 and 1982 IRSIA has increased 28 per cent in the industrial sector and 13 per cent in agriculture, yet decreased 6 per cent in fellowships."

"When you see all these numbers — you see there is something wrong in Belgium."

Biotechnology

Cooperation can succeed

BELGIUM is strong in basic molecular biology. It also has some strong pharmaceutical and chemicals companies. The two are getting together under the wing of IRSIA, Belgium's applied research council, in an innovative, unified scheme which breaks new ground for IRSIA, which normally puts a single company together with a single university group when dealing with universities.

Three years ago the council faced a rash of proposals from companies that were keen to start up in biotechnology. But they all wanted to associate with the same laboratories — the good laboratories. They had seen, says IRSIA advisor Dr W. Degriek, that "something was going on in the universities" and they wanted to join in. "They all needed to take the first step on the learning curve, and they counted on the universities for help."

So IRSIA called them in and told them to take the first steps together. "We said: you must all do the same first phase, and we will bring in only the best specialists from the universities — this will be cheaper and better than the individual projects you've proposed and you will all profit from it."

IRSIA did not restrict the programme to the companies that had applied, but launched a big campaign and added others. The universities then made a global proposal covering most of the companies' needs. A "biotechnology club", now with 33 member companies, was established. Members can introduce their own projects, but they have to show some "solidarity" with the ongoing, "more or less basic" research, preparing the future, training young people in that area.

"So we realized two goals in the same formula: we avoided overlap, and we created a new and larger movement", said Degriek. IRSIA has been pleased with the results and has repeated the exercise in five areas of biotechnology: in fermentation, monoclonal antibodies, genetic engineering, protein engineering, and production

of methane by immobilized enzymes.

Research directions are guided by three-monthly meetings with industry, and IRSIA already has two patents out of "really high-level research."

IRSIA, excited by its success, sees this as a new system of support, which they could apply elsewhere. They call it the "common trunk" — from which branch specific company projects. "It works really well for brand new technologies, where everyone works really well for brand new technologies, where everyone still has to learn, and there is no competition yet."

Royalties from patents are to be distributed through contracts on which IRSIA claims to have done "marvellous work". The contracts are very complex, because IRSIA has had to link the interests of multinationals to those of very small Belgian companies that have completely different markets and possibilities. "You have to marry them somehow. We think that's been one of the successes, having been able to define a contract linking up that very inhomogeneous club."

IRSIA started one project in fermentation in 1981; the formula became more precise in 1982; and the last project, in protein engineering, was added at the end of 1983. There are now three "common trunks" in robotics, two in speech processing, and one in artificial intelligence.

"This is really where we really are bringing the universities in . . . it's never been so clearcut until now . . . before, either a whole industrial sector had to agree on a single project, or it was with one company and therefore rather short-term. Now all the "common trunk" meetings are very active, there's a big exchange of ideas, and the professors have really good support. They get posts, new specialists in their laboratories for a rather long period, not just one or two years."

"We really want", said Degriek "to make the building blocks for the next generation". □

Science's crisis

The logic of application

To understand Belgium one needs perspective. A source of perspective is the Belgian national council for science policy, CNPS, which has just prepared its most important document yet — a set of proposals for solving the Belgian "crisis" in science (it is not too strong to call it that) over the next ten years.

But every perspective implies a viewpoint; and some leading fundamental scientists in Belgium consider that CNPS is too beholden to industry, and too favourable to applied science, for the health of basic research. As circumstantial evidence, the complainants cite an attempt to set up a council of the "scientific aristocracy", including Nobel Prize winners Christian de Duve and Ilya Prigogine, to advise CNPS on the needs of basic scientists in Belgium. This committee of the greats was allowed to die after a two-year trial.

As a result, CNPS — and its president, Professor R. van Geen, ex-rector of the Flemish free university of Brussels and an applied physicist — has become the only effective scientific voice in or near the Belgian national government. So if CNPS were blessed, it would be significant. Moreover, CNPS wields its influence over a government already inclined to think that basic research can look after itself.

However, the accusation does not quite hold up: van Geen and his CNPS are not going to be willing scapegoats for the troubles of basic science in Belgium, although they certainly put an emphasis on application in their 10-year proposals.

So what of the composition of CNPS? With 34 members, it is designed to cover all interests in science and technology, and it must also cope with the linguistic divisions

. . . every researcher cannot do whatever he or she wants . . .

in Belgium. Those apart, CNPS represents four groups: the research establishment (rectors of universities and directors of research); the grass roots of research (young researchers in universities, government laboratories and private industry); the leaders of industry, including banks, some big companies, some small ones (the capitalists, as van Geen puts it); and trade unions and cultural groups. This broad council advises the science policy minister Philippe Maystadt ("a very clever man", who is also government budget minister).

Unfortunately, at the time of writing the CNPS recommendations on policy for the next 10 years are unpublished, unavailable and politically sensitive because, says van Geen, of the "objective" fashion in which CNPS has treated the two communities problem. But he was prepared to talk about its broad outlines.

The first difficulty with Belgian science is

money, the report says. Government spending on science was cut dramatically in 1977, and there has been no recovery. The universities were especially hard hit by the 1977 cuts — "It's the Thatcher policy...". University budgets are still decreasing. "In three of the six universities, they've stopped taking the journal *Physical Review*! Which is unthinkable!"

The proportion of gross national product spent on research in Belgium is too low — at around 1.5 per cent (0.6 per cent by government, plus another 0.9 per cent by industry). Belgium is trailing in the government sector, as a result of cuts in government spending in the face of the economic crisis. But in its 10-year report CNPS argues that Belgium needs to make an effort "precisely because of the economic crisis, not in spite of it...". The greatest difficulty in Belgium is to get this point over: "politicians often consider that research is a luxury, a dispensable item".

Minister Maystadt recognizes the problem, says van Geen, and has a plan to raise the proportion to match other countries in five years. Plans can fail, of course, and in any case the emphasis in this new initiative remains on applied research projects.

But the result of present poverty is a brain drain: "We have very good universities and scientific education — and we're a country known all over world for the quality of its scientists. It's a pity that we can't support them here...".

The CNPS report, however, does do more than cry "more!". "Every researcher cannot do whatever he or she wants" says van Geen: "research today needs more than a pencil." So CNPS suggests there should be a *selective* policy in fundamental research. The policy should be based on finding a proper equilibrium between fundamental and applied research.

The selection would focus on "centres of excellence", which van Geen defines as "those places that have the people, the equipment, and where the science is relevant". The term "relevance", of course, implies selections; but other air can enter the CNPS system, as CNPS is also aware that such centres can age and atrophy. "If you only do science in centres of excellence, at some point you will have none", as their science becomes obsolete, says van Geen. "We have that today with the nuclear fission research centre at Mol. Thirty years ago it was at the frontier of physics, now it's rather out of date."

So as well as support for the centres of excellence, there must be money — "local" budgets, van Geen calls them — to stimulate "trial, background research". For example, van Geen says that Belgium needs a centre for laser research. But if Belgium had not found the money for supporting those doing the initial work on lasers, there would be no core for a centre of excellence. So, van Geen freely admits, a management policy for basic science needs care.

Policy makers must also be prepared to wind down some centres — for example

nuclear fission research — the report points out, to make room for solid state, modern optics, new materials, micro-electronics and biotechnology. Scientists individually must also be flexible. "I had a policy of high flexibility as a university rector — I think it's not only possible but essential." A scientist needs mobility among fields of research; and among institutions (moving among industry, schools, universities, government); and geographical mobility.

The CNPS ten-year forward look, says van Geen, shows by example that really creative developments in science occur when some overlap occurs between disciplines. It is not sufficient to put a physicist *near* a biologist, they need to work on the

same problem, the report argues. But van Geen admits some problems with a directive policy on mobility, flexibility, overlap and centres of excellence. "You must decide *who decides* about the new fields. There you must be very careful. I don't like a 'Russian' system where someone — a bureaucrat — says OK you're doing physics but next week you'll do maths and then two years later biology. You must put people in the right place; and give them also the means to be flexible, the means to learn new fields."

This, then, is the van Geen/CNPS approach to science policy in Belgium; and the reader must decide for himself or herself whether it leaves enough room for basic science to breathe. □

Regionalism

Increasing divide worries centre

THE latest, unpublished report from CNPS — the Belgian national advisory council for science policy — pulls no punches on the "community problem".

Some 55 per cent of the population are Flemish, and 45 per cent Walloon. They speak very different languages, which mark out the history of Europe: the Walloons, in the south, speak the Latin language, French, and the Flemish speak Dutch, which is close to German. The languages differ greatly, and speakers on each side have great trouble learning the other's language.

Moreover, regionalism is increasing. Nineteen-eighty was a turning point, when the national government devolved funds and powers, including policy on applied science, to new regional "executives" (so named to distinguish them from the local "governments" of a true federal system like those of Canada or Germany) plus a separate executive for bilingual Brussels.

Amidst this complexity CNPS has asked the national government to clarify the funding system for science. "Science policy is very difficult now in Belgium", says CNPS president Professor R. van Geen. "Some people want a Flemish and a French science policy — but scientific

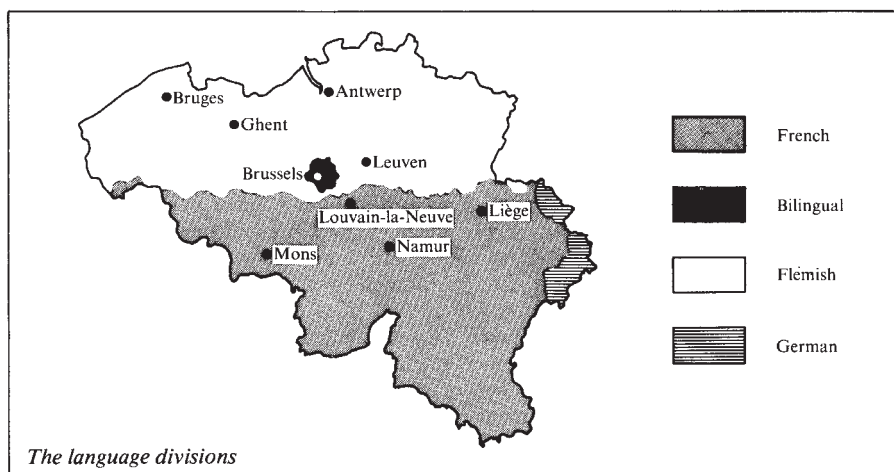
research is a universal thing."

For example, take the government's science policy office (SPPS, science minister Maystadt's executive arm). SPPS is still national. But on both sides of the country there are demands that it be split in two parts.

But, pleaded van Geen, "You must be careful in what you say, as I hope the new equilibrium [between national and regional forces] will help and not hinder research".

"We try to create a balance [in the unpublished CNPS report, which advises on policy for Belgium's next ten years of science] but it is very difficult." The topic is "so electrically loaded today that you have to be very careful. Please report this carefully", said van Geen "as we don't know what the equilibrium state will be". It is "not impossible" that Belgium will split into two nations ("I don't want that — I say that clearly"). In the present circumstances, "fission" could arise out of some political or economic accident.

The community problem has flared up lately for four reasons: first, population. For a long time the French dominated both numerically and politically, but now the Flemish have become numerically dominant and are demanding a redistribi-



bution of money, industry, jobs and political power. Today the Walloons feel they are the victims, when for a century the reverse was true.

Second, Belgium is going through an economic crisis. In the past, community problems have been solved by throwing money at them, yielding equally to "the four forces": Flemish, French, Catholic, and non-Catholic (which means agnostic, rather than protestant, in Belgium). But now Belgium has to decide where to put the little money it can spare: it cannot afford equilibrium.

Third, Flanders is undergoing a rapid economic expansion, with green-field sites for new industry and a more docile population (in terms of strike record) than Wallonia, weighed down by big, troubled

industries like coal and steel.

And fourth, regionalism is increasing everywhere in Europe: the Basques, Brittany, Scotland for example.

So far there has been no serious violence over the regional issue in Belgium — but it could happen. Belgians look in fear at Northern Ireland. And there is a possible flashpoint: a small community of 4,000 people in Belgium which is contested between both sides: a small bit of land which has become a symbol. To judge by some newspapers, the major political problem in Belgium today is that the mayor of this community cannot speak Flemish. There has been some violence there — guns, bombs — but so far without victims. "Violence is increasing", says van Geen, "I just hope it will not explode." □

(technical) colleges and the three universities. "The problem was that only one university [Leuven] was training the people that industry needed", said van Nuffel. To transfer this knowhow the executive took four lecturers from outside Leuven and put them through a very intensive, specially-designed course at Leuven in very-large-scale integrated circuit (VLSI) design. The following year these four trained another thirty. In the third year (which begins this September), these thirty will go back to their institutions (universities and technical colleges) and introduce courses on VLSI.

Moreover, in order to intensify the training, the executive has set up a computer network based in Leuven but linked to every teaching institution. Students in colleges throughout Flanders will use it for real-time, computer-aided chip design, using the Leuven software and equipment.

The expected result: that every year from 1986 onwards Flanders will be producing 200–300 engineers with specialist experience of custom VLSI design. VLSI research will be concentrated in a new BF 2,300 million inter-university centre at Leuven: IMEC. A company (Inventive Systems) has been established to promote chips in industry. And Flanders has given 49 per cent backing to Bell-Antwerp (an offshoot of ITT) to set up a custom-chip production plant at a total cost of BF 3,000 million. The plant is to be operational in 1985, and will have the capacity to make an estimated 4 per cent of the needs of Europe. Around 40 per cent of the production will go to ITT, 60 per cent for outside sale. The objective in backing Bell-Antwerp was to give Belgium some strategic independence in the chip market, but van Nuffel could not estimate what fraction of Belgian demand the factory might satisfy.

The executive has worked out similar programmes for materials and biotechnology, but these are still at the planning stage. In materials there is a study concerning the setting up of a laser applications centre; and in biotechnology the executive was expecting the results of a study on a communal, time-shared pilot plant in May. (The plant would be either in Brussels or Ghent.)

Eventually, the regional executives hope to get more money to play with, as certain national laboratories and funds are regionalized (often against their will). For example the national applied research council IRSIA had just been officially one-third regionalized: a third of its budget divided between Flanders and Wallonia. Flanders will get 60 per cent of that one third, and IRSIA's advice on the distribution of that fraction in Flanders will be advice to president Geens rather than to the national government. Says van Nuffel: "it makes no sense, say, if IRSIA supports traditional industry while we support new technology". Thus IRSIA and other institutions will increasingly feel the pressure of regional politics. □

New technology

Flanders sets the pace

FULL of bounce, the Flanders regional executive, in just its third year of operation, is leaping into high technology.

High technology is political: the president of the executive, Mr Gaston Geens, an economist by training, has grasped technology as the lever that will lift Flanders above the rest of Belgium and establish it as a state within a state. And it is all happening very swiftly, after the law of August 1980 that regionalized a measure of responsibility for economic policy and applied research. (Fundamental research and the basic funding of the universities remain national.)

The Wallonian executive has seen the challenge set by the breathless Flemish executive, and is also making strong efforts — particularly in biotechnology — but it has the harder task. Wallonia has the old industry, Flanders the new, and moreover Flanders is going through a kind of spiritual rebirth, emerging from a century and a half of French domination.

It may be, of course, that Flanders will pull the whole of Belgium with it, and the regional competition will prove highly creative; but meanwhile everyone in Flanders knows what high technology means for the region and its politics — independence — and there is almost no public or trade union opposition to the changes, even though, for example, rampant robotization of Flanders factories is creating no new jobs and eliminating some old ones.

Flanders did have some old industry, mainly in textiles, and that has suffered in the general recession like Wallonia's steel and coal. But, claim Flanders officials, the Flemish textiles industry is now automated and competitive again.

The officials also say that Flanders now accounts for 70 per cent of all Belgian exports, compared with Wallonia's 25 per cent. (The rest comes from the Brussels region.) Moreover, last year there was a 21 per cent increase in investment in Flanders,

says the executive, whereas the total investment in Belgium is decreasing.

Ford, for example, is investing some BF 10,000 million (£125 million) to automate its Flanders factories. Employment levels will be unchanged, but, says the executive, if it did not invest, employment would fall with competitiveness.

"The people accept this better here than in Wallonia", says Luc van Nuffel, microelectronics adviser to Geens. Recently, there was a one-day general strike in Belgium, in protest at the economic crisis. The strike was better observed in the French region than the Flemish, and — claims van Nuffel — if the unions were not national, there would have been no strike in Flanders at all.

It is against this kind of background that the Flemish executive is backing the new technologies — and it has been able to move fast by making use of the many reports of the national advisory council on science policy (CNPS). The executive has moved nowhere more rapidly than in microelectronics. In this, and other developments (the executive has three programmes, on new materials and biotechnology as well) the executive is attempting to move in harmony with the three universities of the region: the Catholic University of Leuven, the Flemish Free University of Brussels, and Ghent.

However, the executive believes in concentration of its resources (which total 7 per cent of the whole national government budget) and so it is proving a force for selection, which has suffered in Belgium because of the usual balance that had to be struck between the "four forces". In microelectronics, for example, the executive focused on Leuven, but in such a way as to draw in the other universities in an inter-university scheme.

The Flanders microelectronics education plan is almost literally exponential. Flemish electrical engineers have been, and will be trained in 14 industrial

Microelectronics

Silicon Valley comes to Belgium

PROFESSOR Roger J. van Overstraeten speaks with hardly a pause, and with constant intensity: it is easy to see how he has established — in less than 20 years after graduating from the University of Stanford, California — an extraordinary microelectronics laboratory in his native Belgium, a laboratory which now almost flies free on industrial contracts, depending on the government for only 20 per cent of its budget.

However, that proportion is about to change: the Flemish regional government is investing capital of BF 2,300 million (£30 million) and annual expenditure of BF 1,000 million (£12 million) to expand Overstraeten's group into an inter-Flemish-university laboratory: IMEC (the inter-university microelectronics centre). Reflecting general amazement at such expenditure in the straitened circumstances of Belgium, IMEC has earned the title: "Superlab".

The nucleus of IMEC will be van Overstraeten's group ESAT (electronics, systems, automation and technology) which he started in 1965 as part of the electrical engineering department of the (Flemish) Catholic University of Leuven. ESAT is highly renowned in its field. According to the research vice-president of the Dutch-based multinational Philips, ESAT's work on computer-aided design of chips puts it second only to Berkeley, California. ESAT has already spawned a clutch of successful small companies in the United States and Europe, and is highly sought-after for consultancy work.

ESAT started slowly, with a staff of just eight people in 1970. "That's when we really got a start. We had proved that it was possible to do some original work in microelectronics in Belgium. . .", and as a result the group won one of the first handouts from Prime Minister Théo Lefèvre's "concerted" action" programmes, by which he hoped — and



Van Overstraeten — boss at "Superlab"

succeeded — to improve the financial and competitive position of some of Belgium's top groups.

As say many of Belgium's researchers, the concerted action cash was extremely important. At ESAT, the improvement was something like an order of magnitude. By 1975 ESAT had 60 people, when it merged with another laboratory to reach 85. Its present 150 is "saturation" for the space and facilities available — hence Superlab.

ESAT works in three areas. The most

important, "integrated systems", deals with everything to do with integrated circuits, including ESAT's strong group in computer-aided design (which started early, in 1969), and a group dealing with the physics and chemistry of chip manufacture.

"We have developed 5 micron CMOS technology, 3 micron CMOS technology, and transferred it to industry. It's unique that a university laboratory should go so far [towards industry] says van Overstraeten. "But we did it partly because there was no Belgian company in the field — we did it to prove what Belgium can do". Now there are five Belgian chip companies, some of them headed by ESAT staff.

Van Overstraeten's ambition is national: why else would he have come back from Silicon Valley? And he set up in university rather than industry "because I think that education and training are extremely important — and training is still our main objective. Every year at ESAT we train 100 electronic engineers in this field. [Superlab will double or treble that number.] And all of them have the chance of designing integrated circuits."

"So I think that a laboratory like this should be between university and industry. It's not a typical university laboratory, but it's not an industry laboratory either."

Belgium will also soon have MIETEC, a joint venture between Bell-Antwerp and the Flemish executive, due to begin producing custom chips in October 1985. "I don't think a company like that could have started in Belgium without the people we have trained."

ESAT also works in systems and signal processing. More and more this field will require custom-designed integrated circuits, van Overstraeten believes. Part of the signals research is fundamental mathematical work in cryptography. For the implementation of a code is always a specific integrated circuit, "and more and more it gets difficult to separate these things . . . a mathematician alone can end up with something that would be difficult to implement in 20 years," whereas codes are needed on a 5–10 year time scale.

The third area of ESAT work is photovoltaics — based on technology similar to that of integrated circuits. ESAT developed a screen printing technology for making solar cells, 4 years ago, as an application of existing integrated circuit technology. The process is materials-efficient, and is now the property of a new Belgian company, Photon Technology, doing well according to van Overstraeten. . .

There are other laboratories like ESAT on a scientific level, van Overstraeten admits. "But if I take integration with industry, there's none like it. It's different from Stanford, and from Berkeley. There the industry is to hand, here we had none." And ESAT has international links with industry in the United States, Switzerland, Germany, Holland, France, the United

Professor van Montagu's local industry

MANY Belgian basic scientists are now turning to applied science — but some with more success than others. One of the more successful ones is Professor Marc van Montagu, plant geneticist at Ghent. Van Montagu has contracts with many companies, but has also set up his own — just started last year: Plant Genetic Systems (PGS). (Van Montagu's Ghent colleague, Professor Walter Fiers, also runs a company — an offshoot from Biogen, a genetic engineering company: Bioghent.)

Such enterprise is not unknown among molecular biologists — but van Montagu's PGS does stand out for one unusual policy: "We mostly hired people locally — in Belgium. We've seen other groups take people from all over the world, but they just end up with a collection of competent but competitive people, without a common spirit. So we wanted to take a different approach, to get the spirit going first, to show we can do it, and then give the team additional help by injecting foreign people if it needs them."

PGS will "apply our techniques to en-

gineer plants to improve their commercial value, for example for resistance to viral and fungal disease, to increase nutritive value. Those are the things we think we can do now . . . and also a lot of work on soil microorganisms. And we have started in association with the university of Brussels (ULB) a programme on protein engineering."

The students don't object to working with industry, says a "pleased but surprised" van Montagu. "Most of the younger students find it challenging to do applied research . . . I used to think that they would object for political, ecological, or other reasons." They don't.

His group has a patent on some Ti plasmid vectors "but we decided the money should go to the university. We don't know if it's making money yet. It takes a long time."

PGS was started with BF 400 million (£5 million) and must live from the interest on that capital plus contract research. The goal for the end of 1984: to be in balance on the current account. □

Kingdom — “that’s quite unique too” claims ESAT’s director.

But ESAT still needs government support: “Our funding now is quite strange, we are living mainly on projects — only 20 per cent comes from the university and fixed funding — that makes life very dangerous” — particularly when electron beam apparatus can cost \$3 million.

ESAT has been supported by three concerted action programmes, by national programmes, by the EEC, IRSIA, the science policy programming service, FNRS, and several ministries. The university pays 25 salaries. “But most important is that the university allows us this openness, and that they support us in getting funds.” Van Overstraeten says he is not bothered by the lack of university posts. He can make do on soft money. And the people who work in his laboratory are sure of a job in industry.

And now comes Superlab, IMEC: it will be an important laboratory, with 150 permanent staff and 100 people working on projects in design and technology. (ESAT’s present budget is about a third of IMEC’s projected spend.) IMEC, available to the universities of Leuven, Brussels (Flemish side) and Ghent, and technical colleges, will also have closed-circuit television, so the courses and seminars at

IMEC can be followed at the other centres, with audio feedback for questions. “It’s a new formula for a research laboratory, an inter-university centre combined with interaction with industry”.

IMEC could not have been founded without government investment. The investment in people is so huge, a university could not do it. But the *national*

. . . Belgium may sound complicated. . . but finally things seem to work out quite well. . .

government could not have begun IMEC — because it required a choice between regions and a focusing of funds.

IMEC is thus “a Flemish thing, but the research will be European — and we’ll work for French-speaking companies, there’s no problem there”. Nevertheless, says van Overstraeten, “If I look at Maystadt [the national science minister] he’s doing excellent things. . . Belgium may sound complicated and it is, but finally it seems to me things work out quite well. . .”.

Flanders is developing fast, the mentality is different, the industry is different, it is a challenge for the French side, but, says van Overstraeten, “I don’t think they’ll stay behind”. □

800 students, created ten years ago, which offers just the first year of university studies. (This is where Luxembourg does its basic research.) The CU first year is intensive, with an amazing student-teacher ratio of 2 to 1, and CU courses are widely recognized by other universities in Austria, West Germany, Belgium and France.

If CU staff, like Seck and his five colleagues in the department of science, want to do research “we have to collaborate with a team abroad”. CU research interests can be listed in total: a collaboration with a French CNRS director working on ectysteroids in insects at the University of Strasbourg has revealed the same ectysteroids in worms; chemistry at the CU has focused on heterocyclic and organometallic compounds, in collaboration with the universities of Metz, Heidelberg and Louvain; the CU physicist works with Centre Hospitalier on medical electronic signals, seeking correlations with disease. And there is one pure mathematician, in a world of his own as far as Seck is concerned. . .

The CU department of science has around 40 teachers, but only Seck and his five colleagues are employed by the CU alone: 20 of the others are upper-level teachers at *lycée*, qualified to teach both at secondary and tertiary level. Then 6 come from foreign universities on secondment, and 10 are professionals such as doctors and engineers.

Research in the CU is done by the 6 full-timers. “But we’ve recently won a new freedom — to release some of the secondary school teachers for several days a week.” That gives a total of about 12 man-years per year of research capacity at the CU, except for the teaching load.

It is a small team, but, says Seck, his laboratories “are very well-equipped for the subjects we’ve chosen” with apparatus like a Mossbauer spectrometer, NMR and X-ray diffractometers, and the department is promised a new building.

Thus basic research in Luxembourg proves viable, at least while it remains linked to the science going on in surrounding countries, and while it is not so ambitious as to outstrip the country’s capacity.

The only trouble is that Luxembourg science is like an unfinished painting, a few dabs of industry, public and private research that could do with a little coordination. “We have to grow together.” Aware of this, in 1983 the members of the research advisory council demanded more power to coordinate and fund Luxembourg’s science. The council would not continue without that power, the members threatened.

But the government — since 1979 led by the Christian Socialist (right wing) party that has ruled Luxembourg almost continuously since the nineteenth century — called the bluff and simply failed to renew the council’s mandate. At the time of writing the council’s future is unclear, but Seck has hopes for new developments after the June national elections. □

Luxembourg

Old industries in decline

RESEARCH in Luxembourg? It does exist. Luxembourg boasts a long-dead Nobel Prize winner and in 1977 the Grand-Duchy began to expand its tiny research capacity. But Luxembourg’s government science is on a very small scale — just 12 researchers in the basic sciences, who really are only nominally independent: as they recognize themselves, they depend absolutely on collaboration with groups abroad. Perhaps only 0.1 per cent of the Luxembourg gross national product is spent on both basic and applied research (but there are no official figures).

The Grand-Duchy has only the population of a large London borough — 366,000 people — and no full university, although it has 3,000 in university education, mostly abroad. But it had the coal and iron ore to justify a major steel industry (Arbed), which ten years ago employed half the working population (now the fraction is plummeting to a quarter). Arbed has a research laboratory with 30–40 staff. Luxembourg also hosts the Goodyear tyre company’s European research laboratory (with some 150 researchers). And some smaller companies also do research in Luxembourg, mostly on metals and materials.

But Luxembourg really could do more research, and needs to in order to be prepared for the new industries that must follow steel. And, indeed, in 1977 a Mr Robert Krieps, minister for cultural and scientific affairs in the — rare in Luxem-

bourg — socialist coalition government, began a slight movement in that direction. Krieps created a number of new institutions for research, among them an advisory council for research policy headed by chemist Professor Pierre Seck. One of the biggest new creations, says Seck, was a small-scale teaching hospital where some important research is done. And Krieps created institutes for technology (technical colleges) that do some applied research.

But despite 3,000 potential students, Luxembourg does not have a complete university. What it does have is the beginnings of one: the Centre Universitaire (CU) with



Pierre Seck — not quite alone.

Fourier's law obeyed — official

Analysis of a mechanical model characterized by deterministic randomness (chaos) allows verification of elementary principles of heat conduction. But it may have other value.

THOSE who hold that the reductionist programme is a mistaken attempt to account for all phenomena in terms of elementary processes usually look for evidence to support their case in the behaviour of living things. They may be well advised, it seems, to pay closer attention to a long-familiar part of classical physics and in particular to the theory of heat conduction.

The problem, as a little reflection will show, is that there is still no unambiguously unobjectionable way of calculating from the laws of dynamics as applied to atoms and molecules the most elementary principle underlying the phenomenon of heat conduction — the notion that the rate of heat conduction between two parallel plane surfaces in some medium is proportional to the temperature difference between them, widely known as Fourier's law. From this simple statement follow the differential equations which specify the time-dependent variation of temperature or of heat flux in conducting objects of all kinds. Calculations based on Fourier's law have naturally been widely used in tasks as different as the inference of temperature distribution within the Earth and the design of industrial heat exchangers. So in what sense can Fourier's law be less than well grounded in physical principles?

The familiarity of Fourier's law no doubt blinds us to its non-trivial character. It is not, however, difficult to construct other plausible phenomenological schemes to describe the rate of heat conduction through material objects. The most obvious modification is that suggested by the process of radiative heat transfer: each element in a body at a temperature different from absolute zero might give rise to an isotropic heat flux which is simply a function of the temperature of the element. For small temperature differences, Fourier's law would be approximately true, and while the rate of heat conduction through a fluid from some solid object would awkwardly be a function of the temperature of that object, it might in practice be hard to distinguish such a variation of conduction away from a source of heat from the variation with temperature of the heat conductivity of media of all kinds.

But surely, even schoolboys will ask, has not this issue long since been dealt with? Heat conduction through gases is familiarly dealt with by kinetic theory, with results entirely in accordance with Fourier's law. For more general fluids, it may be

necessary to take account of more complicated treatments of transport in kinetic systems along the lines originally proposed by Enskog, but the objectives are the same — to calculate the rate of transport of kinetic energy from the distribution of the momenta of particles and the chance of collisions between them. In metals, on the other hand, it is more prudent to start from the likely excitation of conduction electrons into states of higher energy, and to allow for the interruption of free electronic motion in such a state by the vibration of the underlying lattice. Although most of this is intricate, in principle it is straightforward. So why should Peierls (in 1961) have written that the derivation of Fourier's law from elementary dynamics is "one of the outstanding unsolved problems of modern physics"?

Peierls' remark derives from his own work in the 1950s on the interaction between lattice electrons and the spacings of the underlying lattice. That the interaction may sometimes mean that a regular lattice breaks up into consecutive insulating patches or, alternatively, will permit the efficient transport of electrons in the forms of "solitons" is now part of the familiar understanding of conduction in solids, superconductivity in particular. The deficiencies of the theoretical foundation of Fourier's law consist, from this point of view, in the way in which all attempts to calculate heat transport (and other transport processes) from first principles rely, at some level, on an averaging process.

This is the starting point for a calculation published by Joseph Ford and Franco Vivaldia (Georgia Institute of Technology), Giulio Casati (Milan) and William M. Visscher (Los Alamos) at the end of last month (*Phys. Rev. Lett.* **52**, 1861; 1984). Their first task is the choice of a deterministically random (or chaotic) mechanical system with which to model a solid heat conductor. Such a system must be simple enough to be calculable, at least numerically, yet free from exceptional modes of behaviour that might vitiate the modelling of thermal conductivity and yet sufficiently complicated to be vaguely reminiscent of the real world. With fashionable but needless whimsy, the authors settle for what they call a "ding-a-ling" system — a one-dimensional array of hard spheres similarly anchored by symmetrical restoring forces to fixed lattice points with equal freely moving spheres sandwiched between them. At the end of

the array are two thermal reservoirs, which communicate with the conducting array by means of similar freely moving spheres, which if accepted into the reservoir are promptly returned with a velocity specified by the velocity-distribution appropriate to the corresponding temperature.

The model works straightforwardly. The stiffness of the lattice-bound particles has to be great enough for chaotic motion to supervene. What happens is that the oscillating spheres act as relatively unresponsive transducers for energy carried by the intermediate freely moving spheres. The rate of energy transport can be estimated from the rate at which energy emerges from the high temperature end of the system, or from the equal rate at which it arrives at the other. The numerical calculations have been carried through fully only with very short linear chains, with two and four anchored spheres respectively. The result, cheerfully, is a constant coefficient of thermal conductivity (within ten per cent or so). The argument has, however, been made more plausible by means of a calculation of next-nearest neighbour correlations (between neighbouring anchored spheres) for a more general system, from which again a constant thermal conductivity is derived.

So far, then, so good. Here is a simple dynamical system with many of the recognizable characteristics of a heat-conducting solid which does indeed transduce energy in accordance with Fourier's law. Many people will no doubt now be tempted to embark on generalizations of this simple model. Two dimensions? Even three dimensions? Excessive zeal of that kind would be misplaced. What Casati *et al.* have done is to demonstrate that a dynamical system does indeed behave as would be expected under the influence of a random input of impulse. In passing, they have also been able to show that in strictly mechanical systems such as these, the exceptional dynamical solutions, the putative solitons, are no great encumbrance if the vibrational frequency of the anchored spheres is sufficiently great. The other side of this coin is that such models might indeed be used for studying energy transport in, say, superconducting materials. Whether reductionists should be alarmed by all this is quite a different matter, although some of them may be dismayed that this may be that hitherto elusive problem for which only computer solutions are attainable.

John Maddox

Palaeontology

Biased record of early life

from Malcolm Walter

IF an expedition to Earth from some other planet were to be mounted, and if the budget restrictions were such that only two samples could be collected to determine the history of life on Earth, it is probable that the palaeontological advisors to the expedition would urge the collection of a black chert to represent the first few billion years of biological history. Suppose, then, that the collectors travelled first to an island in Hudson Bay, where they gathered a sample of the 1.8×10^9 year old black chert¹, and then to Shark Bay in Australia or some similar arid shore², where they gathered a sample of extant life from the intertidal zone. When the two samples were analysed on return from Earth, it would be concluded that there had been no evolutionary progress for at least 1.8×10^9 years, and that the Earth was still in 'the age of cyanobacteria'.

Palaeontologists are aware of biases resulting from selective preservation. Disproportionate attention to cherts is not a result of naivete on the part of the palaeontologists who work on Proterozoic sequences, but is a consequence of the very success of the studies concentrated on cherts³. Ever since the seminal discoveries in the 2×10^9 year old gunflint chert of Ontario⁴, black cherts have been known as a good preservative for microbial cells, and consequently many studies, including that of Zhang Yun⁵ on page 547 of this issue, have focused on them. As a result, our view of the Proterozoic history of life is probably grossly distorted. Zhang Yun adds to the small but steadily growing list of described microfossil assemblages which represent much of our information about life in Early Proterozoic times ($1.65\text{--}2.50 \times 10^9$ years ago). Although there are techniques, such as the study of stromatolites and isotope geochemistry, that give us different sources of information about ancient life¹, microfossils are preserved cells and the most direct evidence we have. Of the various sources of information available to palaeontologists, microfossils have received by far the most attention. They are preserved in very finely crystalline silica, chert, and as a result of circumstances not yet well understood, most Proterozoic microfossiliferous cherts appear to have formed in the marine intertidal environment.

The attention to a limited set of palaeoenvironments has consequences in biostratigraphy as well as palaeobiology. Any attempt to use chert microfossils in biostratigraphy is greatly limited even before it begins, unless we can find cherts that preserve samples of microbial life from more than just the intertidal zone. Like a few other assemblages, Zhang Yun's could

represent a microbiota that was unique to the Early Proterozoic. The first reaction to this new report is to view it as an encouragement to biostratigraphers, but caution is needed. As in the case of most of the other examples, including the original one from the famous Gunflint Iron Formation, the Chinese example comes from rocks whose depositional environment has not been determined by modern sedimentological techniques. The fact that its microbiota are distinctive may therefore signify nothing more than that it comes from a distinctive palaeo-environment (Zhang Yun suggests the shallow sea in a region of volcanic activity) that has, so far, only been sampled in the Lower Proterozoic — a familiar problem to palaeontologists.

Proterozoic microfossils are also found in shales, where they are called acritarchs, but there they are studied by different techniques and usually by different scientists from those who work on cherts^{6,7}. Many of the shales were deposited in off-

shore marine environments and, as a result, contain a sampling of the marine plankton of the time. They can be expected to have been more diverse than the intertidal microbiota, not having been limited by the environmental extremes that characterize the intertidal zone. Shales should therefore provide a fuller record of evolution through the Proterozoic, and the material should provide the basis for a successful Proterozoic biostratigraphy. A combination of the palaeobiological approach that has been applied to cherts and the empirical biostratigraphical approach of the acritarch workers will give us a true picture of life in the Proterozoic, and provide the basis for sound biostratigraphy. Such work has begun⁸. □

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Oncogenetics

Progress in malignancy

from Miranda Robertson

TWO rare tumours of childhood are proving to be a remarkable source of insight into the genetic steps by which cells may progress in malignancy. The tumours, retinoblastoma and neuroblastoma, have two significant features in common. First, they are both derived from embryonic precursors of nerve cells, which normally lose the ability to divide once they have differentiated; and second, both may be associated with chromosomal deletions. These two facts have been incorporated in the following hypothetical schema. The chromosomal deletion contains a gene that is normally responsible for turning off the embryonic phenotype, including the ability to divide; in its absence, embryonic genes remain active and the proliferative capacity of the cell is retained. It has been suggested that some of the cellular proto-oncogenes may be genes that are normally active only during embryogenesis; and with this in mind, Lee *et al.* have looked for an active oncogene in cells from ten fresh retinoblastomas. They find that the *N-myc* gene is transcriptionally active in all of them and amplified in two¹. At the same time, Schwab *et al.*, who originally discovered the *N-myc* gene², report that in retinoblastomas *N-myc* amplification is associated with late stages of tumour progression³, and thus may have prognostic implications.

N-myc is an oncogene by an unusually complex chain of inference. It was first detected by Schwab *et al.* in the amplified DNA of a number of neuroblastoma cell lines through its limited homology to the *c-myc* cellular proto-oncogene. Thus its identification as an oncogene rests on its relationship to a known oncogene and its presence in DNA that is amplified in tumour cells. Its activation in a second neuroectodermal tumour — retinoblastoma — strengthens the evidence implicating it in neural tumorigenesis.

The first step in the development of neuroblastoma and retinoblastoma must occur before the differentiation of the central nervous system is complete, and indeed may be inherited. The hereditary cases are often associated with chromosomal abnormalities involving the deletion of material from chromosome 1 in the case of neuroblastoma⁴, and from chromosome 13 in the case of retinoblastoma⁵. This is believed to predispose individuals to develop the tumours, but does not make it inevitable, presumably because a normal allele of the deleted gene remains on the intact chromosome. In the case of retinoblastoma, there is now very good evidence that the second step in tumorigenesis is the inactivation of the normal allele⁶. (The mechanisms by which this may occur in retinoblastoma and other childhood

tumours have been the subject of two recent articles in *News and Views*^{7,8}.) Whether the inactivation of both alleles is sufficient for malignancy is not known; but there is some evidence for activation of a member of the *c-ras* oncogene family in a neuroblastoma cell line⁹, suggesting that it may not be.

What is the significance of the transcriptional activation and amplification of *N-myc* in retinoblastoma and neuroblastoma cells? Nothing is known about the function of the *N-myc* product, but *c-myc*, with which it has some homology in the 5' coding region, is implicated in the control of proliferation in several different cell types¹⁰, and it is possible that *N-myc*, whose expression seems to be confined to cells of neuroectodermal origin¹¹, has an analogous role in neural cells. In neuroblastoma moreover, the level of transcriptional activity seems to be correlated with the degree of differentiation of the tumour cells¹¹, which can vary considerably within a single tumour. Schwab *et al.* have shown by hybridization *in situ* that *N-myc* expression is higher in the less differentiated cells.

The association of *N-myc* amplification with tumour progression is suggested by investigations by Brodeur *et al.* on a series of 63 tumours taken from untreated patients at four different stages of the disease, defined chiefly by the extent of dissemination of the tumour cells. Brodeur *et al.* find no amplification of *N-myc* in 15 tumours at stages 1 and 2; but amplification in half of the 48 tumours from stage 3 or 4 patients³. The prognosis for patients at these later stages is usually extremely poor, two-year survival being 10–30 per cent, compared with 75–90 per cent for earlier stages. If the correlation with *N-myc* amplification holds up, it will be important to discover whether the absence or presence of the amplified gene distinguishes late-stage patients who may survive from those who will not. Such a prognostic tool could help with difficult decisions on the more heroic forms of therapy in advanced disease.

More fundamentally, further research on the interactions of the several genes now implicated in the genesis of neuroblastoma should help to illuminate the mechanism of neural differentiation and its disruption in tumorigenesis. □

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Particle physics

How neutral are atoms?

from Frank Close

UNIONIZED atoms are electrically neutral because the negative charges on the electrons precisely balance the positive charges on the nuclear protons. This concept of balancing is so deep-rooted in our scientific culture and so naturally presented in school classes that we hardly give it a second thought. In fact it is really rather profound; why should electrons and protons, which seem to be independent forms of matter, know so precisely the magnitude of each other's electrical charge?

An answer that has suggested itself to many is that ultimately there is but one type of matter: electrons and protons are siblings. If so, this would be the culmination of a long search for an underlying unity in nature. At the beginning of the century scores of atomic elements were thought to be the templates of nature's infinite variety. Then it was realized that three 'elementary particles' — electron, proton and neutron — were the common building blocks of all atoms. Later the proton and neutron were discovered to be built from more basic particles, called quarks. Now we believe that quarks and leptons are the two fundamental families of matter. Yet the precise identity of their electrical charges hints that they might not be totally unrelated, and in the grand unified theories (GUTs) uniting the electromagnetic, weak and strong forces, one finds that quarks and leptons are siblings. This startling result is a consequence of the electrical charge equality between the electron and proton. But how sure are we that these charges are identical, and what consequences would ensue if they were different?

Einstein had suggested that the electron and proton charges (Q_e , Q_p) might not be precisely equated and that:

$$Q = (Q_e + Q_p)/Q_p \sim m_p/m_{pL} \sim 10^{-19}$$

where m_p and m_{pL} are the proton mass of 1 GeV and the Planck mass of 10^{19} GeV respectively. Twenty-five years ago Bondi and Lyttleton noted that if $Q \sim 10^{18}$, electromagnetic repulsion would dominate gravitational attractions among hydrogen atoms on a galactic scale and the expansion of the Universe could thus be explained within Newtonian mechanics (*Proc. R. Soc. A* **252**, 313). Since that time the hot big bang model has been established so we may now take this as a possible cosmological bound on $Q < 10^{-18}$ for hydrogen.

The first precision test on the electrical neutrality of atoms was performed by A. Picard and E. Kessler 60 years ago (*Arch. Sci. phys. nat.* **7**, 340). In their experiment, all ions were removed from a vessel containing a gas. As the gas leaked out of the vessel, they measured the change in the vessel's electrostatic field. If $Q \equiv 0$ there should be no change. Their limit for Q was

$< 2.2 \times 10^{-19}$ for carbon dioxide. Other experimenters have used this technique on nitrogen and argon and have typically obtained limits $< 5 \times 10^{-20}$, consistent with the standard folklore value of 'zero' but perhaps not a definitive falsification of Einstein's hypothesis. Indeed J.G. King applied the Picard-Kessler technique to hydrogen and helium and obtained a small negative charge for hydrogen ($-2.5 \pm 1.5 \times 10^{-20}$) and a small positive charge for helium of $(4 \pm 2) \times 10^{-20}$ (*Phys. Rev. Lett.* **5**, 562; 1960). Subsequently, King has claimed, but not to my knowledge published, details of a null result for H_2 and for He where $Q < 6 \times 10^{-21}$.

What about elementary particles that are believed to be uncharged? A nonzero charge for the neutron can be sought by passing neutrons through an electrostatic field. The most precise limit appears to be due to R. Gahler *et al.* (*Phys. Rev. D* **25**, 2887; 1982): $Q(n) = (-1.5 \pm 2.2) \times 10^{-20}$. For the neutrino, the value comes from astrophysical arguments. If neutrinos are electrically charged, then photons can convert to neutrino-antineutrino pairs and affect the rate of stellar evolution. We know that our Sun has survived for over 5×10^9 years and this places an upper limit on the rate of energy loss by a possible neutrino pair production. J. Bernstein *et al.* find that this limits the neutrino charge: $Q(\nu) < 10^{-13}$ (*Phys. Rev. B* **2**, 1227; 1963).

If, as generally believed, electromagnetic interactions are described by a local and causal field theory, then electric charge must be conserved. Thus in the β -decay of the neutron into proton, electron and neutrino, $Q_e + Q_p = Q_n + Q_\nu$. So if one accepts charge conservation, it provides a better limit on Q_ν than the astrophysical argument. But there is nothing that requires each side of this relation to equal zero.

The question of the electrical charges of fundamental particles has recently been investigated by Okun *et al.* of the Institute of Theoretical and Experimental Physics, Moscow (*Phys. Lett.* **138B**, 115; 1984). In electrodynamics, and in the electroweak theory of Glashow, Salam and Weinberg, the electric charges are not fixed: proton and electron could have arbitrary charges. The reason for this lies in the mathematical group structure of the theories. Electrodynamics is based on the gauge group $U(1)$, and the electroweak theory on $SU(2) \times U(1)$. The $U(1)$ factor gives a freedom to the charges of the matter particles which form the representations of the group. The β -decay equality must be preserved, but the magnitude of $Q_e + Q_p$ is arbitrary. However, in GUTs based on compact semi-simple groups, such as $SU(5)$, the electrical

charges are determined. If the electrical charge operator is a generator of a semi-simple group, then the sum of the electrical charges of the particles in a multiplet of the group vanishes. In the simplest GUT based on $SU(5)$, the fundamental quarks and leptons form two distinct multiplets and hence two separate combinations of charges vanish. Together with the β -decay constraint, this places sufficient constraints to determine the charges of all the quarks and leptons. The electron and proton charges are identical and of opposite sign; the neutron and neutrino charges are both zero.

Leptons and quarks belong to the same multiplet and so group transformations can convert quarks into leptons. As a result, protons, made from quarks, can decay. Searches for proton decay are in progress; the candidate events and lifetime do not appear to be in accord with the simplest $SU(5)$ GUT. GUTs based on other gauge groups are still viable. The gauge group $SO(10)$ is among the best investigated. Here the quarks and leptons fit into a single multiplet and hence only a single set of charges vanishes: the charges are less constrained than in $SU(5)$. Combined with the β -decay constraint one finds an amusing consequence. The hydrogen atom is neutral; the charges of neutron and neutrino are opposite but not necessarily zero. So hydrogen is neutral, but heavy atoms are not if $Q_n \neq 0$.

Do grand unified models require equality of electron and proton charges? Conversely, would a difference in their charges, albeit small, rule out the grand unification programme? Okun *et al.* show that, in principle, grand unification is possible in such an eventuality. However the price is that there must exist a number of Higgs particles (these are predicted to be responsible for generating masses of W, Z particles and the fundamental matter field) so vast that their presence in virtual states would destroy standard low-energy electrodynamics. It is possible to avoid this catastrophe, but even so the models do not look very attractive.

Within the grand unified philosophy, then, it seems we must have $Q_e + Q_p \equiv 0$. The present limits, of the order of 10^{-20} , are tantalizing and it is by no means ruled out that an improvement could bring unexpected results. Indeed, experiments searching for evidence of fractional charge on niobium balls are using balls containing of the order of 10^{20} electrons and protons. If there is an imbalance in $Q_e + Q_p \sim 10^{-20}$ it would lead to a net charge on the ball that could mimic a fractional charge. This would not show up in experiments with oil or smaller metal drops, where as few as 10^{12} protons and electrons are sometimes present. It is possible that null results in these experiments could place strong limits on the electron-proton charge equality. $\square$

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Cardiac physiology

A finger on the pulse

from A.F. Brading

IN the determination of the mechanisms used by the heart to control its intracellular ions and regulate the rate and strength of its beat, the emphasis is shifting from descriptive accounts of factors affecting intracellular calcium to a more mechanistic approach in which the behaviour and control of the various processes involved is under scrutiny. So much was clear from a recent symposium* on the regulation of contraction in cardiac muscle.

The resting level of intracellular calcium is controlled by a combination of intracellular buffering and transmembrane pumps and leaks. Experiments on intact muscle and on isolated sarcolemmal vesicles reported at the meeting brought out the possibility that comparatively simple systems may underly the considerable complexities seen in intact preparations. From measurements with ion-sensitive microelectrodes of the intracellular activities of sodium and calcium and of membrane potential in sheep Purkinje fibres and ventricular trabeculae, in a variety of conditions, the stoichiometry required of a Na-Ca exchange for it to determine the intracellular free calcium has been calculated (H. Fozzard, University of Chicago). Fozzard finds that intracellular calcium adjusts so that the relationship between the sodium and calcium electrochemical gradients is unchanged, and fitted by a coupling ratio of 2.5 with a 'reversal potential' near the resting potential. J.P. Reeves (Roche, Nutley) has characterized an equivalent system in isolated vesicles of cardiac plasmalemma. It consists of a symmetrical exchange of one Ca^{2+} for three Na^+ in a direction determined by the calcium and sodium gradients and the membrane potential. He maintains that in normal resting conditions in the intact heart, the exchange will not be at equilibrium, and will mediate net calcium efflux even during membrane depolarizations to about 0 mV. These results suggest that other processes are more important than Na-Ca exchange in setting the level of intracellular free calcium, although the electrogenic exchange may contribute to the membrane potential under appropriate circumstances.

The rise in free calcium during the contractile cycle involves its entry through the membrane and release from the sarcoplasmic reticulum (SR). Calcium channels and transmembrane calcium currents can now be elegantly monitored with the patch-clamp technique, and R.W. Tsien (Yale University) illustrated the behaviour of voltage-sensitive calcium channels in

normal conditions and in the presence of calcium antagonists and agonists. He suggested that the calcium channel operates in one of three modes, within each of which the channel may exist in one open and two closed configurations, with rate constants dependent on the membrane potential and the mode. Calcium antagonists bind preferentially to a mode in which channel opening is a rare event whilst the calcium agonists stabilize a mode in which the channel open-time is prolonged and the closed-time shortened. The mode existing most frequently in depolarized conditions is characterized by relatively short but fairly frequent channel openings. The interesting possibility was raised that there might be endogenous ligands which could modulate channel behaviour.

Calcium release from the SR is acknowledged to be one of the most important factors responsible for elevating free calcium levels. A. Fabiato (Virginia Medical College) has investigated its importance in mechanically skinned single cells with an exceedingly sophisticated set-up in which changes in the solution around the cell are made in a microprocessor-controlled and precisely timed sequence. The solutions contain aequorin, and its light signal and changes in tension are used to monitor calcium release. It seems that calcium entry during the spike triggers calcium-induced calcium release from the store, and depolarization of the SR membrane plays little part in the release process. Fabiato believes that the calcium is released via a calcium channel which is both activated and inactivated by free calcium on the outer surface of the SR. This means that release is not merely triggered by the level of free calcium but depends also on the rate of change of calcium activity. Inactivation starts at a lower concentration of free calcium than does activation, and with a much lower rate constant. Rapid application of supramaximal calcium levels will inactivate the process before release is triggered, but in the continuous presence of supraoptimal calcium concentrations another process of oscillatory calcium release is triggered (also described by D. Eisner, University College, London, in intact tissues with elevated intracellular calcium) which may trigger arrhythmias in intact heart.

Once intracellular calcium is raised, its ability to activate the contractile machinery can also be modulated. For example, by correlating tension and intracellular calcium activity (measured using aequorin), J. Blinks (Mayo Clinic) showed that, following activation of catecholamine β -receptors, calcium becomes less effective at producing contraction,

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whereas following α -receptor activation, the contractile apparatus becomes more sensitive to calcium. Activation of either receptor results in positive inotropy, but that produced by β -receptor activation is due to cyclic-AMP-dependent increases in calcium entry and calcium release from the SR.

The final meeting session covered clinical aspects. The underlying effects of hypoxia on mammalian hearts and the mechanisms of action of cardiac glycosides and the anti-

arrhythmic agent lidocaine were discussed with reference to the involvement of the mechanisms discussed in earlier sessions. The possibility of developing more useful positive inotropic drugs was discussed by P. Poole-Wilson (Cardiothoracic Institute, London), but the meeting appeared to be somewhat pessimistic about this being achieved in the near future. □

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DNA replication

A twofold amplified view

from David T. Denhardt

How does the mammalian cell accomplish orderly replication of its genomic DNA? Among several hypotheses, perhaps the most popular is the analogy with viral systems — that is, specific sequences in the DNA are activated in a regulated fashion by 'origin-activating' proteins, which cause replication to begin at what would then be considered replicon origins. Recently, a window onto this process has been opened as the result of studies on the replication of amplified, or amplifiable, replicons. In a well designed series of experiments, Mariani and Schimke have demonstrated the amplification of the gene for the enzyme dihydrofolate reductase (*DHFR*) in Chinese hamster ovary (CHO) cells in the course of a single cell cycle (*J. biol. Chem.* **259**, 1901; 1984).

During the normal cell cycle, the *DHFR* gene is replicated once in early S phase. When Mariani and Schimke blocked the entrance of a synchronized population of cells into S phase with hydroxyurea — an inhibitor of ribonucleotide reductase and so of DNA synthesis — replication of the *DHFR* gene was severely inhibited. Upon release from the block, the gene was rapidly replicated. More interestingly, and contrary to expectation, when replication was inhibited by hydroxyurea shortly after the cells had entered S phase, at a time when the *DHFR* gene had been replicated, within 12 hours the gene was replicated again.

The evidence is convincing that, within a single cell cycle, twofold amplification of the *DHFR* gene occurs in a substantial portion of the cell population. What this result suggests is that when DNA replication is inhibited by hydroxyurea part-way through S phase, the cells revert back to their pre-S state. Hence, after removal of the block, the cells initiate replication of their DNA all over again. In effect, at least when the inhibition occurs in early S phase, the cell cycle clock is turned back to the boundary between the G₁ and S phases.

If an explanation for these events is to be sought in terms of 'origin-activating' proteins, one might infer that their stability is enhanced when DNA replication is inhibited to an extent sufficient to permit

them to initiate a second round of replication within the same cell cycle. Programmed stability of an 'origin-activating' protein would readily account for the amplification of the chorion genes of *Drosophila* follicle cells seen in the elegant electron micrographs obtained by Osheim and Miller (*Cell* **33**, 543; 1983).

Reinitiation at previously used origins during the same S phase has also been detected by, for example, Lavi, who demonstrated that when simian virus 40-transformed CHO cells are exposed to

DNA-damaging agents (alkylating agents, aflatoxin) both the integrated viral genome and certain unlinked genes (*DHFR*, the *HLA* locus, c-Ha-ras) are amplified (*UCLA Symp. molec. cell. Biol.* **11**, 659; 1983). Moreover Barsoum and Varshavsky have shown that mouse 3T6 cells, which amplify their *DHFR* genes in response to the presence of the *DHFR* inhibitor, methotrexate, will do so with greater facility in the presence of tumour promoters and mitogenic hormones (*Proc. natn. Acad. Sci. U.S.A.* **80**, 530; 1983).

Support for the idea that there are specific origins of replication in mammalian DNA was reported last year by Hamlin and co-workers (*Nature* **302**, 439; 1983). They studied the replication in synchronized cells of the *DHFR* region in a CHO line carrying a 1,000-fold amplified *DHFR* gene (plus adjoining DNA) and found that replication of each of the 1,000 copies of the amplified DNA started in the same restricted region of the DNA and proceeded away from that origin in a bidirectional manner. Although the origins of replication have not yet been localized to a defined nucleotide sequence, this will surely soon be accomplished. □

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CAMELIA VS CADET

ON her travels in Wonderland, Alice was given the choice of heading in the direction of a Hatter or a March Hare. She chose the latter because "The March Hare will be much the more interesting, and perhaps, as this is May, it won't be raving mad — at least not so mad as it was in March". Lewis Carroll, and Martin Gardner after him in *The Annotated Alice*, were wrong to ascribe such a specific seasonal insanity to the Brown Hare, according to 1,500 hours of observations by Anthony Holley and Paul Greenwood summarized on page 549 of this issue. The mad behaviour of hares is continuous throughout their long breeding season which lasts from January to August, and is no more frequent in March than in any other month of that season. Equally wrong, they conclude, is the traditional belief that the pairs of hares seen madly boxing are both males, and that the boxing represents intrasexual competition. On the contrary, boxing is between a female and an unwanted suitor: here Camelia is seen to be getting the better of Cadet.



Bio polymers

Silken spider chains

from Paul Calvert

SILK is best known as the fibre spun by the larvae of many moths to form a protective cocoon for pupation; most commercial silk is from the domesticated larvae of the moth *Bombyx mori* with some 'wild' silk from the Tussah silkmoths *Antheraea*. A great variety of other insects also make silks, fibres of which find uses as supports, shelters and attachments, but the real silk specialists are spiders. Despite the efforts, in the eighteenth century, of M. Bon of Montpellier, who made himself stockings and gloves from spider silk, it has caught on neither commercially nor scientifically. Research papers on spider silk, such as that by Gosline *et al.* on page 551 of this issue, are therefore rare treasures.

Most silks are proteins, containing a predominance of glycine, alanine and serine. For instance, *B. mori* silk is close to a simple repeating (Gly-Ala-Gly-Ala-Gly-Ser) structure. By comparison, spider dragline silk stands out in having 10 per cent each of proline and glutamic acid. In properties and structure, the silks are first cousins to the nylon fibres. Commercial nylons, however, do not have such a high density of amide units as the silks because it makes for strong hydrogen bonding, which results in a melting point too high for processing and a fibre that is too water-sensitive. A shirt that becomes elastic when wet could be a little discomforting; but for animal silk, hydrophilicity is essential as it permits the fibre to be spun from aqueous solution.

The paper of Gosline *et al.* builds on the work of Work and colleagues who showed there to be a 50 per cent contraction, along with a twofold volume increase, when spider dragline fibres are allowed to swell in water (*Textile Res. J.* **47**, 650; 1977 and **52**, 349; 1982). (The Work papers are worth reading at least for the description of how to milk a spider of its dragline.) This water sensitivity is particularly strange in that the dragline must support the falling spider and yet the twinned lines of the garden spider, *Araneus diadematus*, which can weigh 0.65 g, break at 1 g (see F. Lucas *Discovery* **25**, 20; 1964; Lucas & Rudall *Compreh. Biochem.* **26B**, 475; 1968). Such a close strength specification seems to leave little room for rapid deceleration and none for unpredictable properties.

Gosline *et al.* show that the uptake of water converts the amorphous regions of the fibre to a rubbery state. Silks, like synthetic fibres, are composites of small crystalline regions and rigid glassy amorphous regions. For spider silks, no information on the proportions of the two phases seems to have been published, but most natural and textile fibres are at least 50 per cent crystalline. On swelling in water

the glassy regions become plasticized so that the chains are mobile, and possibly some of the crystals melt. The result is that the structure relaxes, the chains in the amorphous regions coil up and the modulus decreases by a factor of 1,000. If the fibre is stretched and dried, the original properties return. This process is similar to heat shrinkage of synthetic fibres; for instance, drawn polyester fibres, when heated to 240°C, shrink by 75 per cent and the modulus drops 50-fold (M.P.W. Wilson *Polymer* **15**, 277; 1974).

The size of the modulus drop in spider silk is surprising. The final modulus of 10^7 Pascals is more like that of true rubber than that of a partly crystalline polymer — the modulus of a 50 per cent crystalline polyethylene is 50 times higher. This suggests that the water-resistant crystallinity of spider silk is very low and that most of the dry fibre is oriented glassy material. A

reasonable guess would be that the high proline and glutamic acid contents are responsible for preventing much crystallinity developing. This appears strange when the spider could have produced a strong water-resistant fibre of the silkmoth or nylon type. What the spider gains is a fibre that is about twice as strong as *Bombyx* silk and has a very much larger extension (30 per cent) before it breaks. This extension will gradually slow the falling spider rather than there being a thread-breaking jerk. The penalty, apparently, is a rather disconcerting change in behavior in the rain.

Synthetic fibre makers should be impressed by the range of properties attainable within the silk group and spurred in their efforts to control polymerizations to the point where silk-like structures can be duplicated. As a general rule it is very hard to increase the strength of a material without also decreasing the extension to break; the way that both are improved at once in spider silk, compared with silkmoth silk, is impressive. □

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Cell physiology

Cellular site of calcium regulation

from Andrew P. Somlyo

THE concentration of free (ionized) calcium (Ca^{2+}) regulates not only muscle contraction, but also a variety of other cellular processes including glycogen metabolism, secretion, membrane transport and permeability. In muscle, an intracellular organelle system, the sarcoplasmic reticulum (SR), can store and release calcium, and so regulate cytoplasmic Ca^{2+} in the absence of a change in total cell calcium. But what plays the part of the SR in non-muscle cells? The suggestion that it is the mitochondria seems to have stemmed, in part, from the great attention given to mitochondrial Ca^{2+} transport^{1,2}. When exposed to high (unphysiological) concentrations of Ca^{2+} , mitochondria can accumulate very large amounts of calcium phosphate salts, nearly enough to be turned to stone. As known since the time of Lot's wife, this is an eye-catching device, but not conducive to much further activity. Here I shall summarize some of the compelling arguments why opinion is shifting strongly away from mitochondria towards the endoplasmic reticulum (ER) as the regulator of cell calcium.

Subcellular fractions containing ER and showing Ca^{2+} -accumulating activity have now been isolated from a wide variety of tissues³⁻¹⁰. In some instances, contamination by ER vesicles may account for active calcium uptake attributed to some other organelle, such as synaptic vesicles¹¹ and, perhaps, vertebrate retinal discs. The ER

calcium pump resembles the SR system in using ATP (although other high-energy phosphates can substitute) as an energy source, in being enhanced by calcium-precipitating anions such as oxalate and phosphate and in being inhibited by SH-reactive organic mercurials¹⁰. Moreover, a phosphorylated 118,000-molecular weight protein, thought to be analogous to the phosphorylated intermediate of the SR Ca^{2+} -ATPase, has been identified in ER from rat liver¹². The affinity of several isolated ER preparations for Ca^{2+} is sufficiently high ($K_m \sim 0.2\text{--}0.6 \mu\text{M}$) to reduce cytoplasmic Ca^{2+} to the low levels typical of normal resting cells. It has yet to be determined whether there are specialized regions of the ER with a high capacity for storing calcium, like the terminal cisternae of striated muscle SR. The identity of the subfractions of ER largely responsible for calcium-accumulating activity is not known, although it is probably not inherent to the rough endoplasmic reticulum⁶.

The introduction of new techniques has greatly advanced the identification of the ER as the source and sink of intracellular calcium in non-muscle cells. For example, whereas squid axons are sufficiently large to permit the manipulation of their intracellular Ca^{2+} by internal perfusion^{13,14}, for smaller cells, membrane permeabilization by agents, such as saponin, has been used to manipulate intracellular Ca^{2+} . This has allowed investigators to identify the ER as

a calcium storage site, by showing that Ca^{2+} accumulation occurs even at low Ca^{2+} concentrations at which mitochondria cannot accumulate calcium or when mitochondrial calcium uptake is inhibited^{10,15}. Thus ER in liver cells, synaptosomes and macrophages has been shown to accumulate Ca^{2+} at the low concentrations required of a physiological regulator. A more direct approach has been to visualize, through electron microscopy, sometimes combined with electron-probe X-ray microanalysis, the deposits of calcium (or strontium, which acts as a more electron-opaque substitute for calcium) oxalate in the ER of synaptosomes⁵, squid axons¹³, photoreceptor cells¹⁶ and endothelium¹⁷.

For the ER to regulate cell Ca^{2+} , it must have not only a mechanism of calcium uptake, but also one of calcium release. On stimulation of cells by hormones or transmitters, the signal for Ca^{2+} release may be mediated by a change in the properties of the surface membrane, most commonly depolarization, and/or by a diffusible intracellular second messenger. The feasibility of the first mechanism is suggested by the close (about 10–20 nm) proximity of ER subsurface cisterns to the plasma membrane. These structures, which resemble the 'surface couplings' of cardiac, smooth and some skeletal muscles, have been observed in some non-muscle cells^{18–20} and may be present in all eukaryotic cells. There is also evidence that electrical stimulation of the surface membrane of fibroblasts, known to contain subsurface cisternae, can release calcium from an intracellular source, probably the subplasmalemmal ER²⁰. The second possibility, that an endogenous intracellular messenger releases calcium from the ER, has recently received strong support. Thus in pancreatic cells²¹, in isolated hepatocytes²² and in insulinoma cells²³, a phosphatidylinositol metabolite, inositol trisphosphate, has been shown to release intracellular Ca^{2+} from a non-mitochondrial site, presumably the ER. It was further observed that caffeine releases intracellular Ca^{2+} from a non-mitochondrial source in rat nerve cells²⁴, reminiscent of the caffeine-induced Ca^{2+} release from the SR of skeletal muscle²⁵. Caffeine-induced Ca^{2+} release from the ER has also been observed in macrophages⁸, but not in liver¹⁵.

The first inkling that mitochondria might not have a significant role in the regulation of cytoplasmic Ca^{2+} followed the observation that, in the presence of physiological Mg^{2+} concentrations, the affinity of isolated mitochondria for Ca^{2+} is too low²⁶. In the normal cell, Ca^{2+} fluctuates, at most, from 5×10^{-8} to 5×10^{-6} M between rest and maximal activation, while the Ca^{2+} concentration required for a half-maximal rate of calcium transport by mitochondria is 10^{-5} M or higher^{17,26}. Furthermore, in intact cells, the calcium content of mitochondria is relatively low compared with that of the SR and even after sustained

increases of Ca^{2+} within the physiological range in maximally contracted muscle, mitochondrial calcium content is not increased¹⁷. On the other hand there is no question that in the presence of inorganic phosphate (P_i), mitochondria can accumulate massive amounts of calcium and, perhaps, become initial sites of pathological calcification when cell Ca^{2+} (and P_i) rises to abnormally high levels due to cell damage. In injured cells, mitochondria may serve as the final low-affinity/high-capacity buffer for Ca^{2+} , and moderate loading of mitochondria by Ca^{2+} may be an alternative to cell death. It remains to be shown that such cells survive and that mitochondria can discharge their excess Ca^{2+} to the extracellular space via the various surface-membrane-mediated calcium transport mechanisms that must ultimately maintain the steady-state calcium content of cells. One candidate for a naturally occurring 'transmitter' that can release mitochondrial calcium has been identified: sodium can discharge calcium from mitochondria isolated from heart, brain and skeletal muscle, although not from liver, kidney and smooth muscle mitochondria².

What, then, is the physiological role, if any, of the complicated mitochondrial calcium transport system so passionately pursued by biochemists for nearly 20 years? One possibility is that, although mitochondria do not have the capacity to regulate cytoplasmic Ca^{2+} , they themselves can respond to changes in cytoplasmic Ca^{2+} concentrations by producing very small changes in the Ca^{2+} of the mitochondrial matrix space²⁷. A number of the mitochondrial enzymes (dehydrogenases) are sensitive to Ca^{2+} in the range 0.1–10 μM . Therefore, small changes in mitochondrial Ca^{2+} , achievable by even a relatively inefficient (low-affinity) calcium transport system, could have important effects on oxidative metabolism.

While far from complete, the case (even in plants) for the ER as the major intracellular regulator of Ca^{2+} has clearly become much stronger during the last few

years. The lumen of the ER communicates with the perinuclear space where, in some instances, high concentrations of calcium (or strontium) have been demonstrated. Perhaps an ER system, capable of accumulating calcium against a gradient in the nuclear membrane, was acquired with the nucleus when cells became eukaryotes. Unfortunately for biologists, living systems often evolve to use different structures and mechanisms to solve the same problems (photoreceptor systems and contractile regulation are but two examples), and grand unifying schemes with fundamental principles applicable to 'all cells' are rare. In the regulation of cell Ca^{2+} by the ER in all nucleated cells, we may have a rare example. □

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100 years ago

ARRANGEMENTS have been made by the Council of the Scottish Meteorological Society for the completion this season of the Observatory of Ben Nevis. The first portion of the Observatory was, it may be remembered, opened in October last, and since the observers went into residence continuous hourly observations have been made of the conditions of the atmosphere at the top of the Ben, with special reference to temperature, pressure, humidity, and motion. From the discussion of these, and what were daily made by Mr Clement L. Wragge in the summers of 1881 and 1882, by the Secretary, Mr Buchan, the Council have been fully confirmed in the high

expectations they had formed concerning the value of a high-level station, both in its bearing upon general meteorological problems, and also with reference to possible forecasts for the British Islands. The additions to be made to the Observatory will just double its size, and enable the three observers — who during the winter have been considerably cramped in their one apartment — to work under more comfortable conditions. On the south of the present doorway there is to be erected a shelter for tourists. The estimated cost of the completion of the Observatory will be 800l., which is, however, irrespective of a heavy item of charge for conveying on horseback the materials to the top of the hill. It is understood that the cost of equipment and maintenance of the Observatory heretofore has been heavier than was anticipated. The directors intend shortly to make a fresh appeal for funds to the public, which will no doubt be as liberally responded to as was their last.

From *Nature* **30**, 179, 19 June 1884.

Mutations and oncogenes — cause or effect

SIR — In a field as complex as carcinogenesis, it is difficult to distinguish between cause, correlation and consequence. No thoughtful biologist needs to be reminded of the many cancer theories propounded in this century. The current version is represented by the flood of papers on oncogenes and karyotypic abnormalities as the underlying cause of cancer, with amplification in the popular press.

The problem in unqualified acceptance of the claims can be illustrated by an examination of some details of specific and non-specific chromosome changes in cancer. The most thoroughly investigated of the specific or nonrandom changes is the Philadelphia chromosome (Ph¹) associated with over 90% of human cases of chronic myelogenous leukaemia (CML)¹⁻³. However, there are patients with typical CML whose leukaemic marrow cells are Ph¹ negative at first, but become Ph¹ positive later⁴. In one case, Ph¹ negative and Ph¹ positive cells were observed in the same clone of leukaemic cells⁵. The evidence suggests that blood cells remain karyotypically normal but unstable for some time after the initial malignant transformation but provide a milieu favourable for the establishment of the Ph¹ anomaly⁵.

Similar evolution of nonrandom chromosome alteration occurs in sarcomas induced in rats by infection with the Schmidt-Ruppin strain of Rous sarcoma virus. More than 80% of the tumours have a normal diploid stemline⁶. In some cases, all the cells are normal diploid while in others there are a few near-diploid sidelines or pseudodiploids. In older tumours, however, heteroploidy is common and usually involves the specific, sequential addition of three particular chromosomes. Without a careful impression that the chromosomal aberrations are a prerequisite for tumour formation since the predominance of normal diploid cells in early tumours is sometimes not noted when the nonrandom changes are discussed⁷.

Many of the reports of nonrandom chromosome changes or activated oncogenes in tumours are based on studies of cultured lines of the tumour cells. The caution that must be used in interpreting them is illustrated by a study of eight human gliomas⁸, each of which had a unique and broad distribution of karyotypes ranging from hypodiploid to as much as hyper-tetraploid when first placed in culture. Reference sets of frequent karyotypes were established for each of the tumours, only 7 to 25% of the clonal karyotypes were represented in the reference sets. Thus in no case were the cells which were maintained in culture representative of the original tumour, and any conclusions drawn from their analysis could not be applied to the bulk of the tumour cells. All of the examples given demonstrate that progression

within the tumour and selection in culture obscure the relationship between cause and effect.

Chromosome studies have an advantage over molecular analysis of oncogene analysis because karyotypes can be obtained of individual cells, and a population distribution determined, whereas the biological and chemical methods used to identify oncogenes represent an average of the whole of the population. Furthermore, karyotype analysis is technically simple and has been pursued even in clinical laboratories for many years, so it has provided a depth of information, particularly in human cancer, not yet available in oncogene studies.

There are, however, hints that the questions raised about the role of chromosome changes in tumour development have their parallels in studies of oncogenes. Most of the positive reports of oncogene activation derive from transfection studies with DNA from cell lines⁹. (Chemical identification of activated oncogenes in some 50 reported cases of human cancer has been negative⁹). The most prominent cellular oncogenes are related to acute viral transforming genes which are known to give a selective advantage for cell growth in culture. The possibility must therefore be considered that cell structure selects for a minority mutant cell in the original tumour population. The activated oncogene would then be no more representative of the tumour cells than was the clonal karyotype in the glioma representative of the tumour karyotype.

It has recently been shown that a human osteosarcoma cell line which was negative on transfection to NIH3T3 cells could become positive after treatment with *N*-methyl-*V*¹-nitro-*N*-nitrosoguanidine (MNNG), a mutagen and carcinogen¹⁰. The chemically-transformed cells were more transformed in appearance than the original cancer cells and produced more tumours in nude mice. Obviously the "oncogene activation" was an epiphenomenon unrelated to the original tumour. Although this activation may have played a role in the secondary transformation of the tumour cells in culture by MNNG, even this is uncertain, since a similar enhancement of the transformed phenotype in the same cancer cells by another carcinogen was not accompanied by detectable oncogene activation. Many more inconsistencies in the ever burgeoning oncogene literature could be cited to raise suspicion about their purported role in carcinogenesis (see ref. 9). At the most, their role in tumorigenesis appears to be auxiliary¹¹, at the least inconsequential.

As confirmed recently in *Nature*, the initial effects of carcinogenic treatment affect the entire population of exposed cells¹² and therefore cannot be genetic. The rarer, random occurrences which

characterize the secondary stages of transformation¹² can be explained as well by differentiative¹³ as by mutational events. Indeed, the great mass of (largely dormant) evidence which indicates that cancer is the far end of a spectrum of development of cell and tissues^{14,15} seems to be only selectively disinterested to rationalize gaps in mutational theories of cancer. The lack of an adequate mechanistic explanation for developmental processes and their derangement is no justification for ignoring them.

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Oligodendroglia in multiple sclerosis

SIR — The report by Ludwin¹ that the oligodendrocyte proliferates around an injury to mouse brain is of great relevance to previous studies on the neuroglia in multiple sclerosis (MS). Some twenty years ago, we demonstrated proliferation (or at least increased numbers) of oligodendroglia at the edge of active MS plaques^{2,3}. This observation has recently been confirmed by electron microscopy⁴.

Although oligodendroglia are essentially absent from the centre of MS plaques^{2,3,5}, their proliferation at the edge of the lesion suggests either a response to injury or their involvement in repair of the damaged myelin. Ludwin's dynamic isotope study clarifies the controversial issue of the oligodendrocyte's potential to undergo hyperplasia and possible role in repair.

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Archaeomagnetic dating of Santorini volcanic eruptions and fired destruction levels of late Minoan civilization

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Archaeomagnetic dating on the Minoan ash horizons of the Santorini volcano and on fired destruction levels at late Minoan sites on Crete demonstrates that the basal (Plinian) air-fall ash of the first 'Minoan' pumice is contemporaneous with the destruction levels on central Crete, while the higher 'Minoan' ashes are contemporaneous with the destruction levels in extreme eastern Crete. These destruction levels were almost certainly caused by seismic activity, rather than the ash fall. The determination of a time gap between these events lead to a reappraisal of the archaeological evidence and is important volcanologically.

THE excavation of a highly advanced Bronze Age culture in Crete and surrounding islands at the turn of this century, led to speculation¹ that this Minoan civilization was the basis of some Homeric legends and also of Atlantis. Further excavations apparently indicated that the widespread devastation and extensive burning of towns, palaces and isolated villas were synchronous², and that this appeared to be simultaneous with the eruption of the Santorini (Thera) volcano, 120 km north of Crete (Fig. 1). Marinatos³ proposed that there was a direct relationship between the two events and others, such as Luce⁴, have shown how such an association is consistent with features of the Atlantis legend. It has also been shown⁵ how the volcanic activity could account for the Egyptian plagues and events that occurred as Moses was leading the Israelites out of Exile.

The distribution of ash in the Eastern Mediterranean and seasonal wind patterns suggest a summer eruption^{5,6} and Biblical references⁷ also indicate late spring or early summer. The ashfall could thus have directly destroyed growing crops. The ash clouds may have contained fluorine⁸. It has also been suggested^{9,10} that tsunamis destroyed the Minoan fleet, leaving the area open to invasion. However, objections have been raised to such suggestions particularly as they depend on the eruption of Santorini being synchronous with the destruction levels. Unfortunately no dating method is sufficiently accurate to determine the date of either event. Dating by ¹⁴C has given a wide variety of ages^{11–15}—the most widely accepted are between 1700 and 1200 BC, mainly because such a range is broadly consistent with the archaeological evidence¹⁶. This is based on wall paintings in Egypt, contemporaneous with Thutmose III, which depict envoys from Crete attributable to late Minoan IB (LMIB) times. LMIB vases in Egyptian tombs of this age indicate that Cretan LMIB time corresponds with Thutmose's reign, 1503–1447 BC¹⁷.

Acidity layers in the Greenland ice sheet, attributed to pollution of the atmosphere by the Santorini eruption, have a calculated age of 1390 BC¹⁸, while frost damage in tree rings of the bristlecone pine in southern California have been dated as 1626 BC¹⁹. If *Exodus*²⁰ does record the Santorini volcanic events, this was 480 yr before Solomon's fourth year: 1446 BC, although other dates have been suggested, such as 1250 BC (J. V. Luce, personal communication). There is thus no consensus of any one date for the volcanic eruption.

Archaeomagnetism^{21,22} has conventionally been used as an 'absolute' dating method, based on the fact that fired materials

are capable of retaining a magnetic remanence so that the direction and intensity of the geomagnetic field can be determined for the time that the materials were cooling. For periods when the directional and intensity changes are known, such fired objects can be dated by comparing their properties with that of the previous geomagnetic field. Advances have been made in establishing such geomagnetic secular variation records in many parts of the world, with results from Bulgaria²³, in particular, extending the knowledge of the geomagnetic field in southeastern Europe over the probable age of the Minoan eruptions and destructions. However, such records are still sparse, their ages largely based on ¹⁴C determinations which means that no absolute time scale for secular variation during the Minoan period has been established. However, relative dates can be determined. Materials magnetized at the same time in the same region will normally have identical directions and the intensity of the geomagnetic field would also be the same. It was on this basis that the archaeomagnetic study of the Santorini volcanics was initiated²⁴ and extended to late Minoan sites on Crete²⁵ (see Table 1).

Collection and analysis

To establish synchronicity, sufficient samples had to be collected, to allow averaging out of random errors, and had to be measured with great care. The ashes were particularly difficult to sample as most are either unconsolidated or only loosely consolidated. Collection was by the disk method²², in which 2.5-cm plastic disks are glued to the sample, oriented and removed, with the sample attached, for measurement in a version of the Digico magnetometer²⁶. The flat, upper surface of the disk was oriented with an inclinometer and, wherever possible, a Sun compass. Unfortunately some archaeological sites were protected by a roof so that magnetic compass orientation was necessary on some occasions. The individual orientations are considered to be accurate to about 1°, comparable with the instrumental measurement error, so that each sample error is thought to be of the order of 1–2°. The identification of individual components within any one sample is less precise but such errors should be random and are thus reduced by averaging. The ancient intensity of the geomagnetic field in which the geological and archaeological materials originally cooled was determined by a modified Kono and Ueno²⁷ method.

The magnetic minerals were identified using isothermal magnetic properties, X-ray diffraction, Curie temperature analysis, microprobe, thermal and alternating magnetic field studies. It was found that almost pure magnetite was the dominant remanence carrier in both archaeological and geological

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Table 1 Details of Cretan late Minoan sites

Site	Area	Description and location of sampled areas	Usable samples
Malia	Palace (13)	Area to the east of L'entree sud; samples burnt mud-brick from walls of low elevation.	20
	House ($\Delta\alpha$)	Room 7, purification chamber; inner part of NNE-SSW wall. Area 5—'block-wall' running parallel to wall of room 7 (hard 'vitrified' sandstone). Wall to east of rooms 10 and 11. Room 8, small partition mud-brick wall (magnetic compass).	24
Knossos	Stratigraphical museum extension site kiln 2	Upper kiln walls and kiln chamber end of channels: circular sampling distribution (magnetic compass).	26
Sklavokambos Villa		Room 18 to west of small central court (possibly storeroom or kitchen). Interior of east wall dividing rooms 18 and 15. Room 10—burnt mud-brick and limestone samples.	16
Phaestos	Palace	3 areas: (1) area 101 beyond NE area of Palace (burnt mud-brick partitions). (2) Area to north of central court (open paved corridor ⁴¹) vertical burnt plaster walls on east sides of corridor 41. (3) North wall of room 70 to NW of central event (possibly a lustral basin or guard room).	17
Hagia Triada	Palace	Areas near room 8 and 12—east facing wall at bottom of staircase (scala 60), burnt floor at base of staircase to west of room 8, west interior wall of room 8 at top of scale 60, north exterior wall of room 12.	17
Gournia	Town House Ac	Room 16—large floor level mud-brick wall—samples from top to bottom exterior and interior of wall were exposed.	30
Makrygialos	Villa	Samples of burnt mud-brick from low elevation walls (all orientations) and pillar bases. Also floor samples.	38
Palaikastro	Town (Block N)	Burnt sandstone and mud-brick from low elevation walls (block N). Burnt floor from compartment 9.	59
Kato Zakros	Palace	NE annexe of palace, room N; NE-SW wall, pantry LI; staircase of the shrine; inner N wall of pantry, stores L; inner E (NE-SW) wall, pantry store L; inner E (NE-SW) wall; pantry stores LI. Outer S (SE-NW) wall, pantry store L; N wall, room XI E of door; N wall, room XI, W of door; NE-SW wall, room XII (inner) to W of steps: NW-SE wall, room XI, E of door; NW-SE wall, room XV, W side; NW-SE wall room XV, SE corner; N edge of wall extending NE from room XXVI; NE-SW wall, room XXVI (W edge), NW-SE wall, room XXV (treasury); NE-SW wall, room XXV (above archive niches); doorway of N wall between rooms XXVII and XXVI. (All burnt mud-brick.)	49

samples, occurring within rapidly chilled minerals within a glassy matrix in the tephra. Subordinate titanomagnetite ($X = 0.4$; Curie temperature 420°C) occurs as early phenocrysts in the tephra. The magnetic behaviour²⁸ indicated that the remanence was carried by either single domain or pseudo-single domains. The archaeological materials, mostly burnt mud bricks from large *in situ* structures, had been heated to temperatures above the Curie point of their magnetite. However, even unconsolidated charcoal ashes taken from floors (Hagia Triada palace and Palaikastro block N) were found to contain a stable remanence with identical directions to those of the associated burnt mud brick walls.

Results from Santorini

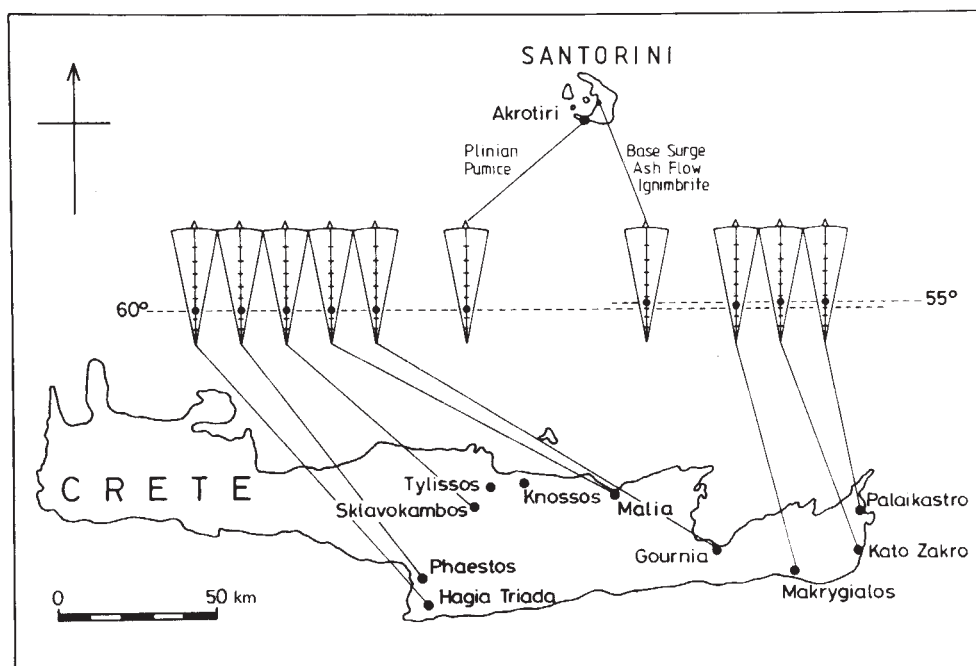
Any interpretation of the magnetic data from the volcanic deposits requires an understanding of how their magnetization was acquired. If they were emplaced at a temperature below that of their Curie temperature, then at least two primary components would be present—one acquired before and one after emplacement. Higher reliance can be placed on directions from a single-component system and palaeointensity determinations from a multicomponent system will be particularly difficult to evaluate. Samples of Minoan tephra were taken from locations on Santorini, ranging from close to the vent (Thera quarry) to those overlying the Minoan city of Akrotiri, 7 km from the former vent (Fig. 2). Analysis of the components, similar to that described by Hoblitt and Kellogg²⁹, yielded emplacement temperatures of 300°C for both the Plinian ashfall and the base surge deposits at Akrotiri, and significantly higher values, $\sim 500^\circ\text{C}$ for both layers, nearer to the vent. The tephra deposits overlying the base surge at Thera quarry were emplaced at lower temperatures, $300\text{--}400^\circ\text{C}$, showing that these originated as ash flows and not mud flows³⁰. The overlying ignimbrites, sampled at several sites on the eastern coast of Santorini, yielded higher

temperatures, above 500°C . (These emplacement temperature results will be discussed in detail elsewhere³¹.)

The presence of two primary components of remanence in many of the ashes overlying the base surge and below the ignimbrites meant that careful analyses were required to separate the two components, and it was frequently the value of the component being removed during partial demagnetization that provided a more accurate definition of the lower temperature component than the component remaining. The ignimbrites and base surge deposits in the quarry were characterized by a single primary component and could be analysed more simply, although these were also subjected to both alternating magnetic field and thermal demagnetization, determination of linearity, planarity, and so on³². These analyses generally demonstrated extremely high stability using any of the standard measures, such as the stability index³³, although 25% of the volcanic samples had to be rejected where the different components could not be adequately separated. Most difficulty was encountered in the ashes immediately overlying the base surge deposits but excellent results were obtained from the Plinian ashfall, the base surge and the overlying ignimbrites. The geological and geochemical evidence indicates that the base surge, overlying chaotic tephra and ignimbrites are essentially part of the same eruptive sequence³⁴ and therefore the less well-defined results from the chaotic ashes make little difference to the final conclusions.

After removal of viscous, short-term components by partial demagnetization, the directions of the component acquired at, and just below, the emplacement temperature were combined for the different volcanic units. This showed that the Plinian ashes were characterized by distinctly steeper inclinations than observed in the overlying ashes and ignimbrites, but all overlying tephra showed mutually consistent directions (Table 2, Fig. 2). The observed difference clearly indicates an age gap between the Plinian and base surge deposits, although this had not been

Fig. 1 Map of the sites and mean directions on Crete and Santorini. The mean directions are illustrated by means of a segment of an equal-area stereographic projection. In this projection the central line indicates north and the inclination varies from zero on the circumference to vertical at the centre. The mean direction is shown as a dot, the size of which is already greater than the 95% precision parameters associated with it. The declinations are more westerly for the projections on the left, but are not very different from those of the other projections. However, the inclinations are statistically steeper on the left than those on the right and this difference is supported by the available palaeointensity determinations (Table 2).



expected from previous geological and geochemical studies³⁴. These showed little or no difference between the composition, refractive indices, and so on, of the Plinian ashes and the bottom of the base surge. Geochemical changes from within the base surge deposits into the overlying ashes were not considered significant as the base surge and overlying chaotic tephra were clearly part of the same volcanic sequence of events. Closer examination of these data indicates that the similarities between the Plinian ashes and the base surge are restricted to the very basal layers of the surge deposits and could be explained if some Plinian ashes were incorporated in the surge as it scoured across their surface. We suggest that geochemical differences may exist, consistent with the magnetic evidence for a significant time gap between the Plinian ashfall and the base surge events. (These new magnetic results differ from those originally obtained

from the Plinian ash and base surge deposits²⁴, but previous sampling methods had restricted the collection to volcanic bombs which are now known not to be characteristic of the main tephra deposits.)

The only archaeological site which could be studied on Santorini was a small hearth structure at Akrotiri (Arvaniti 1 α 1). Unfortunately the roof over the site prevented the use of a Sun compass and the large pithoi nearby may have affected the magnetic orientations. This could account for the higher scatter ($\alpha = 3.6^\circ$) than observed at most other archaeological sites examined in this study. The mean direction differed from those of all of the overlying ashes (Table 2), although this difference was not statistically established at a 95% probability level. However, the palaeointensity determinations on both the hearth and some pottery sherds also indicated an age difference.

Cretan results

Ten Minoan excavations were visited on Crete (Fig. 1), and their final destruction levels were sampled. To detect any deviations caused by anisotropy or magnetic refraction, samples were taken from walls in different orientations and, in the case of kilns, systematically around the wall linings (see Table 1). No evidence was found for either magnetic refraction or anisotropy. The age of each site was ascertained by consultation with local archaeologists and from literature. In all the cases, the fired levels were considered to represent the 'final' Minoan destruction levels. (At Knossos, habitation continued long after the cessation of the Minoan civilization.) These levels have largely been dated on the basis of associated pottery using, for example, Popham's classification³⁵ and are generally regarded as LMIB, corresponding to the presence of a 'marine' style of pottery, and therefore younger than late Minoan I A (LMIA) with a characteristic 'floral' style, although this style persisted into LMIB times. There is controversy about the stylistic classification of some isolated pots and the absence of LMIB pottery does not mean that none will be found or that the extant pottery style at one location was not contemporaneous with LMIB styles as defined at Knossos. At Malia palace, for example, few pots of confirmed LMIB have been found (O. Pelon, personal communication) while LMIB pottery is abundant at Palaikastro.

The presence of single primary magnetic components of very high stability, carried by single or pseudo-single domain magnetite, allowed very precise definition of the directions at each locality. The directions at the sites in eastern Crete (Palaikastro and Kato Zakros) were found to be identical, with the mean

Table 2 Directions and palaeointensities of geomagnetic fields recorded on Santorini and Crete

Site	Mean directions				Palaeointensity (10^{-4} T)		
	Decl. (deg)	Incl. (deg)	α_{95}	No.	Intensity	s.d.	No.
Palaikastro (block N)	355.1	55.1	1.8	59	0.530	0.030	9
Kato Zakro (palace)	355.8	54.6	1.9	49	0.607	0.024	6
Makrygiolos (villa)	354.7	57.5	1.4	32	0.619	0.057	5
Paroxysmal tephra	0.5	54.8	2.0	92	—	—	—
Gournia (house Ac, 16)	356.1	59.5	1.0	24	0.634	0.008	6
Malia (palace area 13)	356.1	59.6	1.4	20	0.617	0.026	7
Malia (house Δα)	356.6	59.9	3.2	22	—	—	—
Knossos (kiln 2 mus. ext)	355.2	60.8	1.6	25	0.615	0.067	3
Sklavokambos (villa)	354.3	59.1	3.9	16	0.692	0.027	5
Phaestos (palace)	356.5	61.1	1.7	17	0.673	0.066	4
Hagia Triada (palace)	353.8	60.1	2.3	17	0.681	0.020	6
Plinian ashfall	359.7	61.1	3.2	40	0.670	0.100	6
Akrotiri (hearth α1)	10.6	57.3	3.6	17	0.510	0.049	6
Akrotiri (pottery)	—	—	—	—	0.578	0.072	5

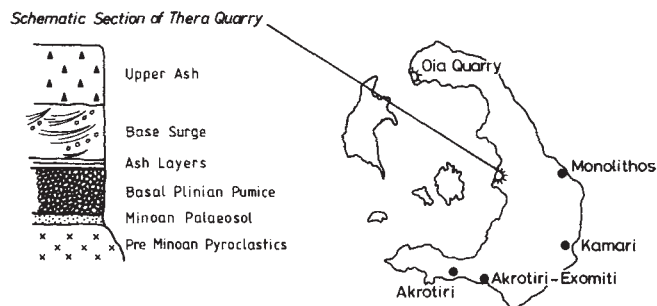


Fig. 2 Sampling sites in Santorini. Most of the tephra sampled were from the sequence exposed in the Thera quarry.

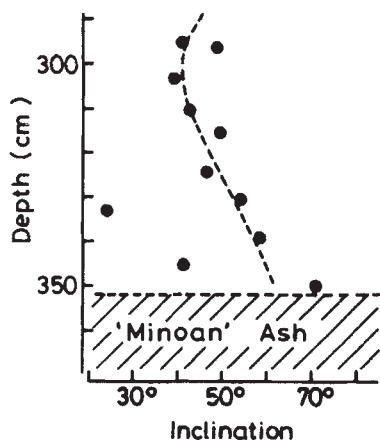


Fig. 3 Changes in the geomagnetic field as recorded in eastern Mediterranean sediments. New magnetic studies²⁵ of the VEMA V10-50⁵ core of the eastern Mediterranean sediments show a decrease in inclination with time in the carbonates overlying the Minoan tephra, consistent with the archaeomagnetic studies on Santorini and Crete.

inclination at the other eastern Crete site, Makrygialos, being only slightly steeper. The remanent directions at sites from central Crete (Malia, Gournia, Knossos, Sklavokambos, Phaestos and Hagia Triada) were significantly different from those in the east, yet were all mutually consistent (Table 2, Fig. 2). The same differences and similarities were then confirmed by determination of the palaeointensity of the geomagnetic field (Table 2), although such determinations are less precise. These results were unexpected as it had been thought that the final destruction levels on Crete would have had similar ages. More surprising was the observation that the directions and palaeointensities determined for central Crete were the same as those of the Plinian ashfall deposits on Santorini, while the directions of the sites in eastern Crete were the same as those of the later paroxysmal tephra deposits on Santorini. The central Crete destruction levels are archaeomagnetically synchronous with the Plinian ashfall and there is a time gap before the destructions in eastern Crete and the paroxysmal eruption of Santorini.

Conclusions

The fact that the Akrotiri hearth appears to have been last fired at some time before the destructions in Crete and also before the ashfall on Akrotiri is consistent with the archaeological evidence^{36,37} that this Minoan settlement was originally destroyed by an earthquake and that partial clearance had been undertaken before it was covered by ash. However, the scatter in both directions and palaeointensities needs to be improved and this is, anyway, only one structure. The determination of reliable LMIA geomagnetic field parameters, therefore, still require examination of an undoubted LMIA destruction level. While a difference in time between the Akrotiri hearth and the

basal Plinian ashes can be considered as strongly indicated, rather than confirmed, there is little doubt about the difference between both the directions and palaeointensities of the Plinian ash/central Crete sites and those of the paroxysmal tephra/eastern Crete destruction levels. However, any evaluation of the actual time represented by the age difference can only be an approximation. If the central Cretan sites and the Thera basal pumice values are combined, giving equal weight to each fired or volcanic level, then their mean direction is 356.1° , 60.0° and the 95% confidence radius is 0.9° . (Knossos is excluded because the only site that was allowed to be sampled was a kiln which may not have been fired at the time of the destruction level—although its similarity in direction and intensity suggests that it was last fired within 2–3 yr of the destruction levels at other central Cretan sites.) Combining Palaikastro, Kato Zakros and the paroxysmal Minoan tephra directions gives a mean direction of 357.1° , 54.8° and a 95% confidence radius of 2.6. The difference in direction is thus 5.2° with the summation of the 95% radii as 3.5° (calculated on a conservative level of analysis as a smaller precision is obtained when these are combined at a sample level). The difference in direction, 1.7 – 5.2° , is small although clear and confirmed by the palaeointensity differences. However, the rate of secular variation of the geomagnetic field is not known for this time. A few records from Bulgaria²³ span the probable age of these events, but lack of information on the precise age of the Santorini/Cretan events and errors in the ^{14}C dating for this time makes any comparison uncertain. New magnetic studies²⁵ of the VEMA V10-50 core of sediments from the eastern Mediterranean⁵ confirm that a major tephra layer, previously attributed to the Minoan eruption on the basis of refractive indices, also has identical Fe:Ti ratios and Mn, Al, Si and Mg content in its magnetic minerals. The overlying carbonate sediments show a decrease in inclination during this period (Fig. 3) but the rate can only be calculated from sedimentation rates which are themselves only estimates. Thus the change in inclination could be some 0.07°yr^{-1} , which is slower than the present-day total change in the regional geomagnetic field of some 0.25°yr^{-1} . The difference in time could be between 100 and 7 yr but the longer time interval seems unlikely, and it is archaeomagnetically unlikely that the time difference could be as low as 7 yr as the observations from the tephra deposits would then show some degree of smearing. A time gap of some 20 ± 10 yr therefore seems to be the most reasonable estimate for this interval.

Implications

The mere existence of a time gap is significant. The fact that it was the central Cretan destruction levels that were archaeomagnetically synchronous with the Plinian ashfall means that the ash itself can have had little direct influence on the Minoan civilization on Crete. The thickness of the ash mantle over Santorini would probably have made it essentially uninhabitable, but only 5 cm of ash is thought to have fallen on Crete^{5,6} and this was mostly in the east, where civilization appears to have continued. It seems almost certain, therefore, that the central Cretan destruction levels were caused by an earthquake—the intense burning presumably resulting from oil spills catching fire, and so forth. Knossos seems to be the only central Cretan site in which habitation continued after seismic devastation, which may imply that this area is geologically less vulnerable to earthquakes. Some 10–30 yr later, the violent eruption of Santorini was probably accompanied by tsunamis and air percussion waves. It seems unlikely that tsunamis were the direct cause of the destruction of the remaining Minoan settlements on eastern Crete as extensive fire destruction is inconsistent with such an explanation. If this 'final' event was only a few years after the Plinian ashfall, with associated earthquakes, then weakened buildings are likely to have been flattened by the percussion air wave, but a time interval of 10–30 yr would suggest that this destruction level was also associated with earthquake activity, rather than being directly caused by the

volcanic eruption. Such earthquakes would obviously be related to the increase in volcanic activity but the firing was again probably due to the seismic disturbance. Firing by invading Mycenaeans^{38,39} immediately after the seismic destruction cannot be excluded, although the extent and intensity of burning is comparable with the central Crete destructions which are not considered to be associated with invasion.

This hypothesis for the end of the Minoan civilization has little direct bearing on postulated similarities with the Atlantis legends or with *Exodus*. Plato's descriptions do not obviously refer to volcanic activity, but are consistent with the second eruptive phase, because the end of Atlantis would then relate to the sudden end of the Minoan civilization simultaneously with the disappearance of the island of Santorini as its 80 km² caldera formed.

Other implications of this study include the evolution of this form of volcanic activity and the determination of tephra emplacement temperatures. Another implication is the way in

which earthquakes near Crete can affect different parts of this island. This seems to be consistent with the evidence for changes in sea-level⁴⁰ which also define separate tectonic units. These, and the archaeological implications clearly require study of Minoan destruction levels at other places and times, particularly at Knossos. It would be especially valuable to extend such archaeomagnetic observations to fired levels within the well-dated Egyptian sequences, as well as Mycenae, to determine both the relative and absolute ages of these events.

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Requirement of either of a pair of *ras*-related genes of *Saccharomyces cerevisiae* for spore viability

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Cells of the yeast, Saccharomyces cerevisiae, containing disruptions of either of two genes that are members of the ras oncogene family are viable, but haploid yeast spores carrying disruptions of both genes fail to grow.

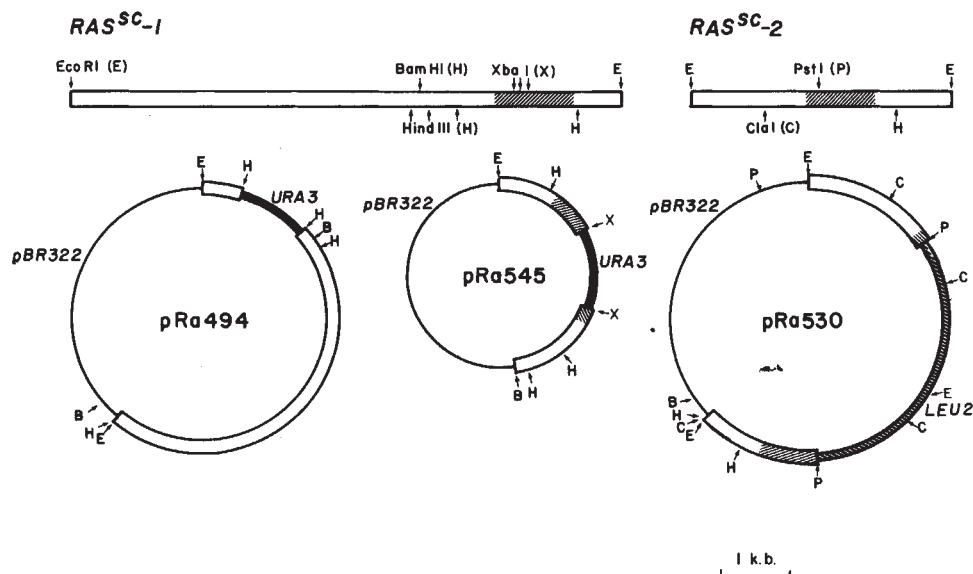
THE cellular proto-oncogenes of the transforming genes from Harvey and Kirsten murine sarcoma virus (*c-ras*^H and *c-ras*^K, respectively) represent two members of a family of related genes called *ras* (ref. 1). Activated forms of these genes have been isolated from some human cancers and/or cancer-derived cell lines that are able to transform NIH 3T3 cells in a transfection assay. Point mutations at amino acid position 12 or 61 (refs 2-9) have been shown to be necessary and sometimes sufficient for the transforming phenotype⁵⁻⁷.

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The *ras* gene family shows extensive evolutionary conservation¹⁰. Cellular *ras* genes have been found in a wide variety of vertebrates. The known range of conservation has been extended to *Drosophila melanogaster*¹¹ and, now, to the yeast *Saccharomyces cerevisiae*^{12,13}. Recently, there have been isolated from the yeast genome two DNA fragments that cross-hybridize with a viral *ras* probe¹³, while a gene located near the yeast actin and tubulin genes also shares homology with the *ras* gene family¹². A set of *in vivo*-labelled yeast proteins are specifically immunoprecipitated by anti-*ras* monoclonal antibodies prepared against the mammalian *ras*-encoded protein, p21 (ref. 14).

The DNA sequences of the two *ras*-related DNA fragments isolated by DeFeo-Jones *et al*¹³ (*c-ras*^{sc1} and *c-ras*^{sc2}) have

Fig. 1 Restriction endonuclease maps of the cloned *RAS^{sc1}* and *RAS^{sc2}* genes and recombinant plasmids used in the gene disruption experiments. The hatched portions of each map correspond to the location of the yeast *RAS* gene. The plasmids were constructed as follows: pRa545, a 1.1-kb *Hind*III fragment containing yeast *URA3* was ligated into the *RAS^{sc1}* gene, at the *Xba*I sites; pRa494, the *URA3* gene was substituted for the *Hind*III fragment containing *RAS^{sc1}* and the *Hind*III fragment adjacent to it; pRa530, a 4-kb *Pst*I fragment containing the yeast *LEU2* gene was ligated into the *Pst*I site within the *RAS^{sc2}* coding sequence. DNA restriction fragments to be subcloned were gel-purified and ligated using published procedures³⁴. The *URA3* gene present in pRa545 and pRa494 and the *LEU2* gene in pRa530 were derived from YEP24 (ref. 35) and YEP13 (ref. 36) respectively. To construct pRa545, the two small *Xba*I fragments present within *RAS^{sc1}* were replaced by a *URA3*-containing *Hind*III fragment. This was accomplished by filling-in the protruding 5' ends of the DNA fragments with DNA polymerase I (large fragment) and then flush end-ligating the fragments together. This recreates *Xba*I endonuclease restriction sites at the junctions.



been determined and are presented elsewhere^{15,16}. Sequence analysis of the two genes, designated *RAS^{sc1}* and *RAS^{sc2}*, reveals open reading frames of 309 and 322 amino acids respectively. The putative proteins encoded by these reading frames are highly homologous to each other for the first 180 amino acids, at which point they diverge. Homology is again found at the extreme C-terminal end of each peptide. A striking pattern of amino acid sequence homology is also found when the putative yeast proteins are compared with the mammalian *ras* protein, p21. The NH₂-terminal 170 amino acids of the yeast and mammalian proteins are >60% homologous. The fact that such evolutionarily divergent organisms exhibit such extensive sequence conservation suggests that the *RAS* gene products fulfill an essential role in cell growth and metabolism. Here we show that the two yeast *RAS* genes isolated by D. DeF.-J. *et al.*¹³ constitute an essential gene family. At least one functional member of the family is required for spore germination and probably for cell growth.

Viability of yeast strains

We have constructed null mutations in the *RAS* genes to test directly the phenotype of cells lacking these genes. These experiments use yeast transformation techniques which have been developed recently (reviewed in ref. 17). In yeast, introduction of exogenous DNA into the genome requires homology between the recipient chromosome and the donor fragment. This requirement allows one to direct DNA fragments to specific sites within the yeast genome. We have used these transformation systems to integrate disrupted *RAS* genes into the yeast genome, excluding the wild-type genes in the process.

Figure 1 shows the restriction maps of cloned *Eco*RI fragments containing *RAS^{sc1}* and *RAS^{sc2}*. Part or all of these two restriction fragments have been used to construct a series of recombinant plasmids containing the wild-type *URA3* or *LEU2* gene. Two mutations were constructed in *RAS^{sc1}* (Fig. 1). The first, contained in plasmid pRa494, harbours a 1.1-kilobase (kb) *Hind*III restriction fragment which encodes *URA3*, substituted for 2.3 kb of *RAS^{sc1}* DNA. This deletion lacks the entire *RAS^{sc1}* gene plus an additional 1.1 and 0.2 kb of noncoding sequence 5' and 3' to *RAS^{sc1}*, respectively. The second mutation, in plasmid pRa545, contains the *URA3* gene in place of two small *Xba*I restriction fragments located within the *RAS^{sc1}* open reading frame (Fig. 1). This deletion spans amino acids 63–150

of the putative *RAS^{sc1}* coding sequence. A single disruption of *RAS^{sc2}* was constructed in plasmid pRa530 by inserting a 3.8-kb *Pst*I restriction fragment, encoding *LEU2*, into a *Pst*I site that corresponds to amino acid 65 in the *RAS^{sc2}* open reading frame. These insertions should therefore disrupt the *RAS^{sc}* gene products.

Plasmids pRa494, pRa530 and pRa545 were digested with the appropriate restriction endonucleases to generate DNA fragments containing *RAS* DNA flanking the *LEU2* and *URA3* insertions. These conditions lead to replacement of the wild-type locus with the transforming sequence as outlined in Fig. 2 (ref. 18). As it is possible that the *RAS* genes encode essential functions, the restricted plasmids were introduced into a diploid strain homozygous for *ura3* and *leu2* (TX2).

To identify those transformants having the desired molecular structure, genomic DNA was prepared from pRa494, pRa530 and pRa545 transformants and analysed by the method of Southern¹⁹ (Fig. 3a). Most of the transformants give hybridization patterns consistent with the simple replacement in one chromosome homologue of the wild-type locus by the null or the disruption mutation. *RAS^{sc1}* is contained on a 2.9-kb *Bam*HI-*Eco*RI restriction fragment. In the pRa545-derived mutations this fragment expands to 3.8 kb, due to the insertion of the *URA3* gene. In the pRa494 mutation the fragment size decreases to 1.8 kb, due to the deletion of the entire *RAS^{sc1}* locus. These structures are shown in Fig. 3b, together with analogous diagrams for the wild-type and mutant *RAS^{sc2}* genes. The hybridization data for transformants of pRa545, pRa530 and pRa494 (Fig. 3a) are consistent with the structures outlined in Fig. 3b. In each case, the transformants contain a wild-type *RAS^{sc1}* allele on one chromosome and the null or disrupted allele on the other.

The phenotypes of haploid yeast strains lacking a functional copy of either *RAS^{sc1}* or *RAS^{sc2}* were determined in haploid meiotic segregants derived from the transformants. The meiotic cell cycle in yeast is initiated when diploid strains are placed on a nitrogen-deficient medium containing a nonfermentable carbon source. The end product of each meiotic event is four haploid spores, collectively called a tetrad. As the *ras* mutations are heterozygous in the diploid transformants, two spores in every tetrad will contain the mutant *ras* allele and two will contain the wild-type allele. The *ras^{sc1}* mutations are associated with *URA3* and the *ras^{sc2}* mutation is associated with *LEU2*, so that *Ura*⁺ and *Leu*⁺ haploid spore clones mark those haploid segregants that lack a functional copy of *RAS^{sc1}* or *RAS^{sc2}*

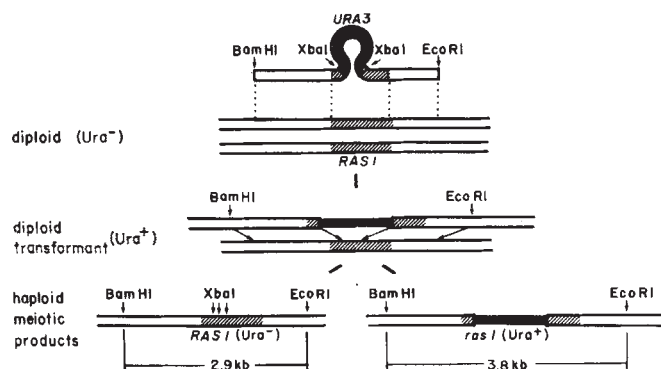


Fig. 2 Schematic drawing of the transformation of diploid strain TX2 with the *EcoRI*–*Bam*HI *RAS*^{sc1} fragment of pRa545. The linear fragment integrates into the yeast chromosome at *RAS*^{sc1}, giving rise to a heterozygous mutation in *RAS*^{sc1}. Sporulation of this diploid gives rise to haploid spores containing either the wild-type or mutant *RAS*^{sc1} locus. Strain TX2 was transformed with linear restriction fragments by the method of Beggs³⁷. Gene disruptions 494 and 530 were created in a similar manner by transforming TX2 with *EcoRI*-restricted pRa-494 or *Xba*–*Hind*III-restricted pRa530 and selecting for uracil or leucine prototrophy, respectively.

respectively; this is outlined schematically in Fig. 2. The recovery of *Ura*⁺ and *Leu*⁺ segregants from TX2-545 and TX2-530, respectively, indicates that the *ras*^{sc1}-1-545 and *ras*^{sc2}-2-530 mutations do not result in cell inviability. The *Ura*⁺ and *Leu*⁺ phenotypes segregate normally through meiosis and *Ura*⁺ or *Leu*⁺ spores have no apparent growth disadvantage on the rich medium used for the analysis. It seems, therefore, that neither *RAS*^{sc1} nor *RAS*^{sc2} is separately essential for ascospore germination and growth. Southern analyses of tetrads from TX2-545 or TX2-530 (Fig. 3a) show that the *Ura*⁺ and *Leu*⁺ spore clones contain only the mutant copy of *RAS*^{sc1} or *RAS*^{sc2}, respectively.

The fact that both *RAS* genes are not required for vegetative growth (mitotic cycle) does not exclude the possibility that both are required for meiosis. To test this, we have constructed diploid strains homozygous for either the *ras*^{sc1}-1-545 or *ras*^{sc2}-2-530 allele.

In both cases these diploids sporulated normally, giving rise to a high proportion of asci with four spores. This indicates that meiosis is normal when the products of one or other of the genes *RAS*^{sc1} and *RAS*^{sc2} are absent.

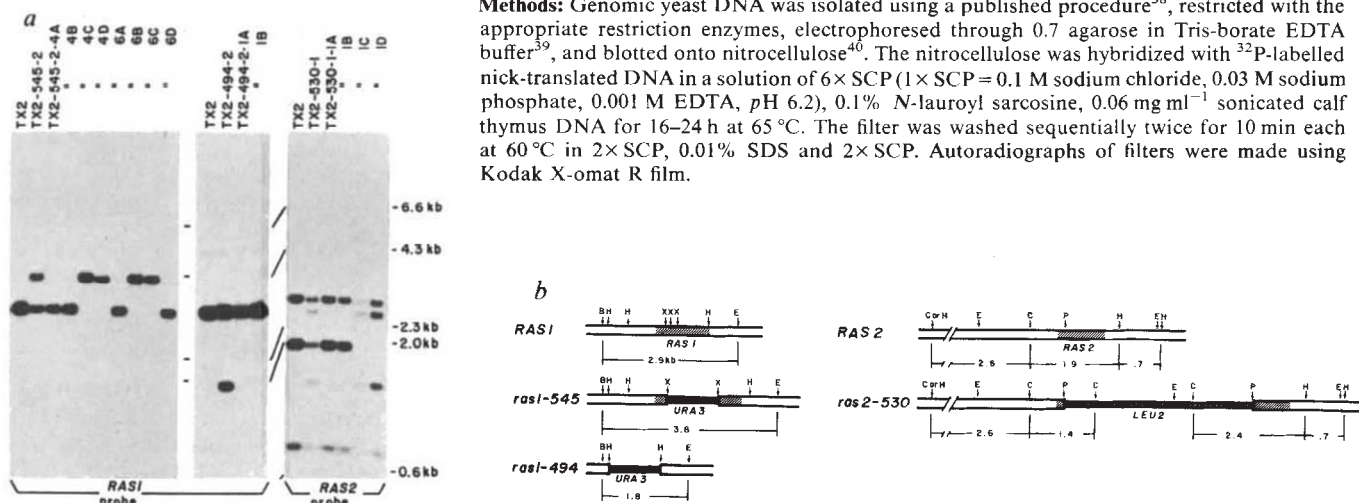
Unlike TX2-545 and TX2-530, transformants derived from pRa494 are heterozygous for a lethal mutation, as only two viable spores in each tetrad of TX2-494 are recovered. Those spores containing the deletion, marked by *URA3*, do germinate, but undergo only one to three rounds of cell division. The fact that the deletion in TX2-494 includes >1 kb of DNA upstream of the *RAS*^{sc1} gene, and the observation that internal disruptions in the *RAS*^{sc1} and *RAS*^{sc2} open reading frames are not lethal, suggests that some essential function separate from *RAS*^{sc1} lies at least partially within the *ras*^{sc1}-1-494 deletion.

Spore viability

Our results suggest that neither *RAS*^{sc1} nor *RAS*^{sc2} separately has an essential function, since no phenotypic change is associated with haploid strains lacking either a functional *RAS*^{sc1} or *RAS*^{sc2} gene. Neither gene alone is absolutely required for either the mitotic or the meiotic cell cycle. But it is possible that the two *RAS* genes may code for proteins with overlapping but not necessarily identical functions. To test this possibility, we constructed a diploid, EG53, that is heterozygous for both the *RAS*^{sc1} and *RAS*^{sc2} disruptions by mating a *Ura*⁺ haploid segregant from TX2-545 (*RAS*^{sc1} disruption) with a *Leu*⁺ haploid segregant from TX2-530 (*RAS*^{sc2} disruption). Tetrad analysis was performed on the resultant meiotic progeny.

Because *RAS*^{sc1} and *RAS*^{sc2} are unlinked (see below), the two genes should segregate independently in meiosis, and give rise to recombinant spores (*Ura*⁺, *Leu*⁺ and *Ura*⁺, *Leu*⁺) frequently. However, no *Ura*⁺ *Leu*⁺ recombinants were recovered in 60 tetrads analysed, although *Ura*⁺, *Leu*⁺ segregants were frequently observed. Moreover, as shown in Table 1, a peculiar pattern of spore germination was observed in the dissected tetrads. Relatively few tetrads with four viable spores were recovered (7/60), whereas ascospore germination in related crosses was good (Table 1). These seven tetrads are parental with respect to *URA3* and *LEU2* in the sense that none of the spores was recombinant. Similarly, many more asci with three viable spores per tetrad are recovered in EG53 (30/60) relative

Fig. 3 a, Southern blot analysis of gene disruptions in *RAS*^{sc1} and *RAS*^{sc2}. The 10 lanes in the first panel correspond to genomic DNA from parent (TX2), heterozygous transformant TX2-545-2 and haploid spore clones from two tetrads of TX2-545-2. Haploid spore clones 4A, 4B, 6A and 6D were *Ura*⁺, while spore clones 4C, 4D, 6B and 6C were *Ura*⁺. The four lanes in the second panel correspond to DNA from parent, transformant 494-1, and two haploid spore clones from a tetrad of TX2-494-1. Only haploid spores containing the wild type *RAS*^{sc1} gene grow into colonies. DNA in the first two panels was digested with *Bam*HI and *Eco*RI and probed with nick-translated *RAS*^{sc1} DNA (*Eco*RI–*Bam*HI fragment). In the third panel the six lanes correspond to DNA from the parent, pRa530 transformant (TX2-530-1), and spore clones from a tetrad of the transformant. DNA was restricted with *Cla*I and *Hind*III and probed with *RAS*^{sc2} nick-translated DNA (*Eco*RI fragment). Spore clones 1A and 1B are *Leu*⁺ while spore clones 1C and 1D are *Leu*⁺. The migration positions of the phage λ -*Hind*III restriction fragment are indicated to the left of the third panel. b, Restriction endonuclease maps of *RAS*^{sc1}, *RAS*^{sc2} and gene disruption present in a. The key to the restriction sites is given in Fig. 1.



Methods: Genomic yeast DNA was isolated using a published procedure³⁸, restricted with the appropriate restriction enzymes, electrophoresed through 0.7% agarose in Tris-borate EDTA buffer³⁹, and blotted onto nitrocellulose⁴⁰. The nitrocellulose was hybridized with ³²P-labelled nick-translated DNA in a solution of 6× SCP (1× SCP = 0.1 M sodium chloride, 0.03 M sodium phosphate, 0.001 M EDTA, pH 6.2), 0.1% *N*-lauroyl sarcosine, 0.06 mg ml⁻¹ sonicated calf thymus DNA for 16–24 h at 65 °C. The filter was washed sequentially twice for 10 min each at 60 °C in 2× SCP, 0.01% SDS and 2× SCP. Autoradiographs of filters were made using Kodak X-omat R film.

Table 1 Lethal segregation patterns of *RAS* disruptions

Disrupted <i>RAS</i> gene	Strain	Viable spores per tetrad					% Germination of spores with <i>RAS</i> disruptions
		4	3	2	1	0	
—	TX2	27	3	0	0	0	
<i>RAS1</i>	TX2-545-2	24	2	1	0	0	48 (Ura ⁺ spore clones)
<i>RAS1</i>	TX-545-3	6	0	0	0	0	50 (Ura ⁺ spore clones)
<i>RAS2</i>	TX2-530-1	16	5	3	1	0	48 (Leu ⁺ spore clones)
<i>RAS2</i>	TX2-530-2	4	2	1	0	0	50 (Leu ⁺ spore clones)
<i>RAS1</i>	TX2-494-2	0	0	65	11	4	0 (Ura ⁺ spore clones)
<i>RAS1, RAS2</i>	EG53	7 (7PD)	30 (29T)	20 (13NPD)	3	0	0 (Ura ⁺ , Leu ⁺ spore clones)

Spore viability of diploid strains heterozygous for *ras* disruptions. EG53, heterozygous for *RAS1* and *RAS2* disruptions, was constructed by crossing Ura⁺ and Leu⁺ haploid segregants from TX2-545 and TX2-530 respectively. In EG53 all asci containing four viable spores were parental ditype (PD) for Leu⁺ and Ura⁺, 29/30 asci containing three viable spores were tetratype (T) and 13/20 asci containing two viable spores were non-parental ditype (NPD). The seven asci with two viable spores that are not non-parental ditype are the result of nonspecific spore lethality.

to the related crosses. In most of these tetrads (29/30), *URA3* and *LEU2* are in the tetratype configuration (one of each parental and one of each recombinant type). By inference, the inviable spore is always the Ura⁺, Leu⁺ recombinant. There is also a disproportionately high number of tetrads with only two viable spores (20/60), and most of these asci (13/20) are non-parental (all of the spores are recombinant) with respect to *URA3* and *LEU2*. Again the inviable spores in these 13 tetrads are inferred to be those with both disrupted *RAS* genes (the Ura⁺, Leu⁺ spores). The results of this analysis demonstrate a striking correlation between the observed lethal segregation pattern and the segregation of the two mutant *RAS* genes, and provide strong evidence that the double mutant (Ura⁺, Leu⁺) causes lethality in haploids. These data, therefore, indicate that the *RAS^{sc}1* and *RAS^{sc}2* genes encode an essential function.

Visual inspection of the inviable spores reveals that not only do they fail to undergo a single round of cell division, but they also fail to form buds. Since bud emergence in vegetatively growing cells and germinating spores occurs early in the S phase of the cell cycle²⁰, this observation suggests that the inviable

spores may be blocked quite early in the cell division cycle. The inviability, however, does not distinguish between a function for *RAS* that is specifically related to the cell cycle *per se* as opposed to a general role in cell growth and/or spore germination. Note also that this lethal spore phenotype is quite different from that observed for the lethal segregants of TX2-494. In the latter case, the spores were able to germinate and undergo one to three rounds of cell division before mitotic growth ceased. This phenotypic difference further supports the notion that the lethal mutation in TX2-494 is not in the *RAS^{sc}1* gene.

RAS^{sc}1 is located on chromosome XV

During the genetic analysis of the *RAS^{sc}1* mutants, we observed genetic linkage between *RAS^{sc}1* and *ade2*, 13 recombination units, on the right arm of chromosome XV. Additional mapping data between *pet17* and the mutant *ras^{sc}1* locus place the *RAS^{sc}1* centromere proximal to *ADE2* (see Table 2). A temperature-sensitive (ts) lethal mutation *tsm8740*, has recently been mapped to chromosome XV, 13 centimorgans from *ade2* (ref. 21).

Table 2 Mapping data for *RAS1* and strain list

Strain	Map interval	PD	Ascus type		T	Map distance (centimorgans)
			NPD			
TX2-494-2	Lethal- <i>ade2</i>	34	0		12	12.4
DCX103	Lethal- <i>ade2</i>	53	0		22	14.7
DCX103	Lethal- <i>ras^{sc}1-494</i> (Ura ⁺)	75	0		0	< 0.7
DCX102	Lethal- <i>pet17</i>	26	2		35	37.3
DCX102	Lethal- <i>ras^{sc}1-494</i> (Ura ⁺)	63	0		0	< 0.8

Strain	Genotype	Ref.
TX2	<i>a leu2 ura3 can1-100 ade2-1 his3 trp1 + lys1</i>	This work
	<i>α leu2 ura3 can1-101 + + trp1 lys1 +</i>	
K381-9D	<i>α spo11 ura3 ade6 arg4 aro7 asp5 met14 lys2 pet17 trp1</i>	30
K392-19D	<i>α spo11 ura3 can1 cyh2 ade2 his7 hom3 tyr1</i>	30
Be326	<i>a tsm8740</i>	21
EG45-7D	<i>a tsm8740 ade2-1 ura3 his3</i>	This work
DCX102	<i>a leu2 ura3 can1 + + + + + + + + + + ras^{sc}1-494</i>	This work
	<i>α + ura3 + ade6 arg4 spo11 aro7 asp5 met14 lys2 trp1 pet17 +</i>	
DCX103	<i>a leu2 + ura3 can1 + + + + + ras^{sc}1-545</i>	This work
	<i>α + hom3 ura3 can1 cyh2 tyr7 his7 ade2 +</i>	
EG53	<i>a leu2 ura3 can1 trp1 + + ade2 ras^{sc}1-545 +</i>	This work
	<i>α leu2 ura3 can1 trp1 lys1 lys2 + + ras^{sc}2-530</i>	

Results of tetrad analysis on strains containing the *ras^{sc}1-494* allele. Genetic procedures are standard³¹ and the sporulation plate media are described elsewhere³². DCX102 and DCX103 were derived from mating haploid segregants of a *ras^{sc}1-494* strain to K381-9D and K382-19D immediately after dissection. This lethal rescue technique is described elsewhere³³. The *tsm8740* allele in EG45-7D was derived from Be326.

Although we find that this mutation is tightly linked to *RAS^{sc}1*, less than 1 map unit distant (unpublished observations), it is not allelic to *RAS^{sc}1*. This has been determined by mating a haploid strain bearing *tsm8740* (EG45-70) with a haploid containing the large *RAS^{sc}1* deletion from TX2-494. (Although spores containing the *ras^{sc}1-494* deletion fail to grow into colonies, they can be mated immediately after germination.) The resulting diploid strain, doubly heterozygous for *tsm8740* and the *ras^{sc}1-494*, is not temperature-sensitive for growth, indicating that *tsm8740* is complemented by the wild-type allele on the *ras^{sc}1-494* chromosome. Similar mapping experiments indicate that *RAS^{sc}2* is unlinked to *ADE2* and *RAS^{sc}1*; its precise location has not yet been determined.

Conclusions

The data presented here demonstrate that *RAS^{sc}1* and *RAS^{sc}2* encode an essential function in yeast. The viability of yeast cells containing disruptions of either of these genes indicates that the function(s) specified by either is sufficient to support both mitotic and meiotic cell division cycles, but that at least one wild-type gene is required for viability. There is no apparent correlation between the rates of spore germination or growth and the disruption of either *RAS* gene.

The inability to recover ascospore clones that contain both mutant *RAS* genes indicates that the two genes encode proteins with similar if not identical functions. Our results do not exclude the possibility that *RAS^{sc}1* and *RAS^{sc}2* have separate functions as we have observed growth rates only under a limited set of conditions. However, these results do show that the *ras*-related sequence identified by Gallwitz and colleagues¹² does not specify the same function as *RAS^{sc}1* and *RAS^{sc}2*, as the sequence fails to complement the defect of the double *RAS* mutant. A situation in which two unlinked genes have nearly identical functions exists for several other yeast genes. Histones H2A (ref. 22) and H2B (ref. 23), and the ribosomal protein p51 (ref. 24) are encoded by two unlinked genes. Disruption of only one of the gene pair generally causes no phenotypic change while disruption of both genes has severe consequences on cell viability. Although untested, it is likely that histones H3 and H4 (ref. 25), glyceraldehyde 3-phosphate dehydrogenase²⁶, and several other ribosomal proteins which have two structural genes²⁷ may fall into this category of duplicated essential genes.

The results obtained from the analysis of one of the *RAS^{sc}1* mutations, *ras^{sc}1-494*, suggests that another essential cellular function is tightly linked to *RAS^{sc}1*. That this essential gene is

not *RAS^{sc}1* is suggested by several lines of evidence. First, the deletion defined by this mutation extends 1.1 kb 5' and 0.2 kb 3' beyond the *RAS^{sc}1* coding sequence, and therefore includes more than just *RAS* DNA. Second, an internal *RAS^{sc}1* deletion in the highly conserved region of amino acid homology does not by itself result in cell lethality. Spore inviability is observed only in cells that carry this internal mutation in conjunction with the *ras^{sc}2* disruption. Third, the lethal phenotype of *ras^{sc}1-494* spores is quite different from that of the *ras^{sc}1 ras^{sc}2* double mutants. Spores containing *ras^{sc}1-494* germinate and undergo one to three rounds of cell division before growth is arrested, whereas *ras^{sc}1 ras^{sc}2* spores fail to divide or initiate bud formation. Although these data do not exclude other explanations, the most likely interpretation is that another essential gene resides in close proximity to *RAS^{sc}1*.

Comparison of the presumed amino acid sequences of *RAS^{sc}1* and *RAS^{sc}2* reveals a very large region of non-homology that is flanked by highly homologous amino acid sequences. The NH₂-terminal 180 amino acids are 83% conserved but the next 100 amino acids show only 20% conservation^{15,16}. The proteins again show a high degree of homology at their C-terminal nine amino acids. Despite this large divergent region, however, the two gene products complement each other. This lack of selective pressure can be accounted for if the diverged portions of the *RAS* polypeptides are removed proteolytically, an event that is known to occur in the C-terminal region of the mammalian *ras* genes^{28,29}. Alternatively, these portions of the yeast proteins may encode a function not particularly dependent on primary sequence. It may be noteworthy that the divergent region of each putative *RAS* polypeptide does show a similar amino acid composition.

Judging from the extreme evolutionary conservation of the *ras* genes, it is not surprising to find that their products have a vital role in cell growth or metabolism in yeast. What does this analysis suggest this role might be? The failure of *ras^{sc}1 ras^{sc}2* double mutants either to germinate or to form buds suggests that the *RAS* gene products are required early in the cell division cycle. These data are also consistent with the proteins being quite rare and/or highly susceptible to proteolysis during the sporulation process. More extensive knowledge about *RAS* function(s) awaits the isolation and analysis of conditional mutations in *RAS*.

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Far-UV and extreme-UV observations of SS Cyg and U Gem from Voyagers 1 and 2

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Recent studies of dwarf novae in the UV and X-ray regions have suggested that much of the energy produced in a dwarf nova is emitted in the far-UV and extreme-UV regions (100–1,200 Å). We report here on observations of SS Cyg and U Gem obtained with the Voyager UV spectrometers (UVS) in the 500–1,200 Å region. SS Cyg has been detected in quiescence at a flux level of 9×10^{-13} erg cm $^{-2}$ s $^{-1}$ Å $^{-1}$ at 1,000 Å. U Gem was not detected in quiescence. No emission shortward of the Lyman limit (912 Å) is detected in either star. Observational upper limits to the extreme-UV flux in the 540–740 Å band from SS Cyg and U Gem correspond to 2×10^{-13} erg cm $^{-2}$ s $^{-1}$ Å $^{-1}$. A far-UV ($\lambda_{\text{eff}} = 1,050$ Å) light curve for an outburst in SS Cyg is presented. The slope of the far-UV flux distribution during the rise to maximum is observed to be correlated with the UV brightness. The nature of this correlation supports the suggestion that the outburst begins at the outer edge of the accretion disk and moves inward. The far-UV flux distributions of SS Cyg and U Gem in outburst were found to be remarkably similar, both exhibit a substantially flatter ($F_{\lambda} \propto \lambda^{-0.5}$) distribution than that observed in the IUE-UV ($F_{\lambda} \propto \lambda^{-2.3}$). This result is contrary to current disk models which predict a continued rise in flux in the far-UV. Evidence for a wind in the Lyman lines of hydrogen is seen in SS Cyg. No evidence of a wind was observed in U Gem.

The UVS¹ on board Voyagers 1 and 2 are nearly identical objective grating instruments having maximum sensitivity in the 500–1,200 Å region and a dispersion of 9.26 Å per detector channel (resolution ~ 20 Å). Previous stellar observations and the absolute calibration have been discussed by Holberg *et al.*². A decline in detector sensitivity towards longer wavelengths establishes $\sim 1,200$ Å as a practical long wavelength limit for observing sources of the brightness of SS Cyg and U Gem. We have therefore restricted analysis of Voyager data to wavelengths shortward of 1,175 Å.

SS Cyg has been observed with the UVS during two outbursts and U Gem during one outburst. Table 1 lists the dates of observation and the characteristics of these outbursts. In quiescence, SS Cyg has been detected at the 2σ level in the 1,000 Å region at a flux level of 9×10^{-13} erg cm $^{-2}$ s $^{-1}$ Å $^{-1}$. U Gem has not been detected in quiescence with the Voyager UVS. During the intervals listed in Table 1, the Voyager scan platform was fixed in the direction of the target, while the spacecraft limit cycle motions allowed the field of view of the spectrometer to wander on and off the target to obtain both source and background signals. Except for the investigation of the change in the slope of the flux distribution in SS Cyg during the rise to maximum on JD 2444373, only on-axis ($\pm 0.02^\circ$) source spectra are discussed here. For that investigation marginally off-axis ($\pm 0.04^\circ$) spectra were combined with adjacent (in time) on-axis spectra. Tests of UVS spectra show that the relative distribution within a spectrum is unaffected by it being obtained off-axis. Thus, this technique results in an improved signal-to-noise ratio within the spectrum at the expense of absolute photometry.

The far-UV light curve of the first outburst (JD 2444373) in SS Cyg is presented in Fig. 1. The light curve was generated by averaging the 925–1,175 Å flux for on-axis spectra. Whenever possible, spectra closely spaced in time were combined. During the rise to maximum this time 'window' was set at 1 h. For the remainder of the outburst, time bins up to 12 h were used. Also plotted in Fig. 1 is the ratio of the flux in the long wavelength channels ($1,090 \pm 40$ Å) to the short wavelength channels (960 ± 40), Ly β is excluded from both bands. The second outburst observed with the UVS exhibited no significant change in flux throughout the 4 days of visual light curve. A mean 1,050 Å flux of $3.00 \pm 0.10 \times 10^{-11}$ erg cm $^{-2}$ s $^{-1}$ Å $^{-1}$ was measured with a $F_{950}/F_{1,090}$ ratio of 0.97 ± 0.05 . The observations of U Gem during maximum also showed no significant change during the 5 days of observation. The mean 1,050-Å flux and $F_{950}/F_{1,090}$ ratio were respectively $1.42 \pm 0.10 \times 10^{-11}$ erg cm $^{-2}$ s $^{-1}$ Å $^{-1}$ and 1.03 ± 0.05 . The U Gem data were also explored for variability coupled to the orbital period of 4.25 h (ref. 3). No variations in flux $> 5\%$ were detected.

The far-UV light curve is similar in shape to the observed visual light curve (J. A. Mattei, personal communication)⁴. The rise in the far-UV seems to be delayed by 0.5–1.0 days with respect to the rise in the visual. Large uncertainties in the times of the UV and visual rise to maximum preclude any discussion of the possible delay. However, it is clear that in the earliest stages of the outburst the rise in flux at shorter UV wavelengths (960 Å) is delayed relative to that at longer UV wavelengths (1,090 Å). This is consistent with the observations of VW Hyi by Hassall *et al.*⁴ and strongly supports their suggestion that the outburst is initiated at the outer edge of the disk and moves inward. During the decline to quiescence there is a suggestion in the Voyager data that the drop at 960 is more rapid than that at 1,090 Å. The difference is less pronounced than that observed during the rise and will require further observation to confirm its existence.

Figure 2 shows composite outburst flux distributions for SS Cyg and U Gem. The far-UV data for SS Cyg are from the

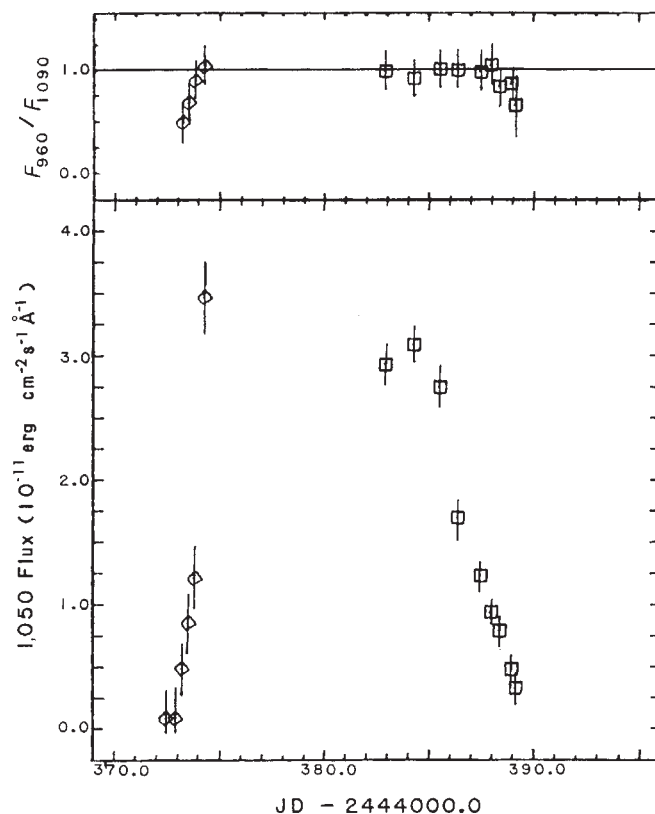


Fig. 1 Far-UV light curve and hardness curve for SS Cyg. Error bars indicate $\pm 2\sigma$. $\diamond$, Voyager 1; $\square$, Voyager 2.

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second outburst observed with the Voyager 1 UVS (JD 2445272). These data pertain to SS Cyg at maximum light and represent a sum of on-axis spectra having a total integration time of 17,280 s. The IUE data for SS Cyg are from A. V. Holm (personal communication) and were obtained on 14–15 June 1978. The ground-based data are from Oke and Wade⁵, obtained on 11 July 1977. All three observations are from long duration outbursts, however, as they are from different outbursts, small adjustments in flux had to be made to get the three segments to match at the overlap points. These adjustments in $\log F_\lambda$ were +0.05 and -0.05 for the IUE and ground-based data respectively. The Voyager far-UV data for U Gem also represent a sum of maximum light, on-axis spectra, with a total integration time of 23,040 s. The IUE data are from Panek and Holm⁶ obtained on 10 October 1980. Although representing different outbursts, no adjustments were made between the Voyager and IUE data.

Examination of the Lyman continuum region ($\lambda < 912 \text{ \AA}$) of SS Cyg and U Gem shows no evidence of any extreme-UV emission. Specifically, in the 540–740 $\text{\AA}$ band Voyager observations establish a 3σ upper limit on the mean flux of $2 \times 10^{-13} \text{ erg cm}^{-2} \text{ s}^{-1} \text{ \AA}^{-1}$ for both stars (see Fig. 2). In the case of SS Cyg such a limit is not entirely unanticipated, as interstellar neutral hydrogen column densities of the order of 10^{20} cm^{-2} have been deduced from soft X-ray observations^{7,8}. Our upper limit for the U Gem outburst is, however, of more interest as a considerably lower H I column density has been inferred for U Gem⁸. We can use our result to establish a lower limit on the H I column density in the direction of U Gem. IUE observations of U Gem⁶ during quiescence have established that shortward of 2,000 $\text{\AA}$ the energy distribution of U Gem approximates that of a 30,000 K white dwarf. Such an object will contribute little to the total extreme-UV flux at 640 $\text{\AA}$, thus any flux at these wavelengths arises from the region producing the quiescent soft X-rays. Extrapolation of this soft X-ray distribution into the extreme-UV region is very uncertain. Depending on the extrapolation used, neutral hydrogen column densities in excess of 10^{17} to 10^{18} cm^{-2} will be sufficient to reduce the expected extreme-UV flux below our limit of detection. Soft X-ray fluxes, however, are observed to increase dramatically during the outburst phase⁸ so that the extreme-UV flux can also be expected to show a corresponding increase. In such conditions, somewhat larger

Table 1 Outburst observations of SS Cyg and U Gem

Star	JD of UVS observation (2440000.0+)	Voyager spacecraft and data rate*	JD of visual rise to maximum† (2440000+)	$m_{\text{v,max}}^\dagger$	Approximate duration of outburst† (days)
SS Cyg	4,372.4–4,374.0	1, L	4373	8.4	17
	4,382.7–4,389.0	2, L, H			
SS Cyg	5,278.5–5,282.6	1, L	5272	8.4	18
U Gem	5,397.7–5,402.4	1, L	5393	9.1	17

* 1 = Voyager 1; 2 = Voyager 2. L, 1 spectrum/576 s; H, 1 spectrum/3.84 s.

† From AAVSO (American Association of Variable Star Observers) light curve charts supplied by Mattei (personal communication).

hydrogen column densities would be necessary to satisfy our observed EUV upper limit.

A comparison of the relative far-UV flux distributions of SS Cyg and U Gem indicate that except for strong hydrogen Lyman series absorption in SS Cyg, the two distributions are statistically indistinguishable. If the UVS far-UV distribution is to be represented by a power law of the form $F_\lambda \propto \lambda^\alpha$, α must be -0.5 ± 0.5 . This is significantly different from the exponent, ~ -2.3 , measured in near-UV (IUE) region. The shape of the distribution does not, however, suggest a power law. Both stars show a noticeable change in slope near 1,000 $\text{\AA}$. Note that the change in slopes seen in Fig. 2 is not an artefact of the Voyager flux calibration. Examination of sources having true power law spectra, such as some very hot O subdwarfs and central stars of planetary nebula, shows that Voyager fluxes produce no discontinuity or change of slope in the power laws which characterize IUE and ground-based fluxes. The most likely cause of this change in slope is line blocking in the dwarf nova disk. A comparison of these flux distributions with those of other objects observed with the Voyager UVS indicate that they are very similar in slope and shape to intermediate temperature ($\sim 50,000 \text{ K}$) subdwarfs or white dwarfs. In particular, this intercomparison of flux distributions reveals that any object with a suggested effective temperature in excess of $\sim 70,000 \text{ K}$ yielded a much steeper slope in the far-UV than either SS Cyg or U Gem. Because SS Cyg and U Gem are at radically different inclinations⁹ (38° and 67° respectively) it is unlikely that this absence of evidence for higher temperatures ($T \geq 50,000 \text{ K}$) could be due

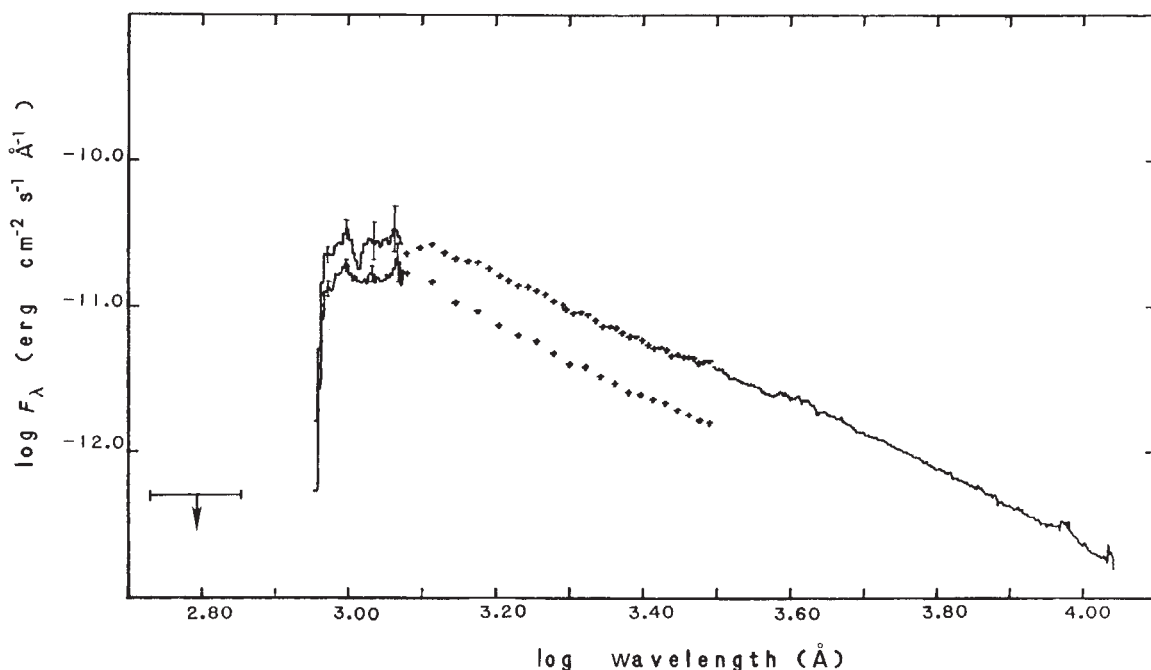


Fig. 2 Composite energy distributions for SS Cyg (upper) and U Gem (lower). The Voyager data (histogram, $\pm 2\sigma$ error bars indicated) are from JD 2445278–83 (SS Cyg) and JD 2445398–402 (U Gem). The IUE data (crosses) are from JD 2443673/74 (SS Cyg, A. V. Holm, personal communication) and JD 2444522 (U Gem⁶). The ground-based data for SS Cyg (solid line) is from JD 2443335 (ref. 5).

to occultation effects. Thus, the inner disk in these dwarf novae must be radically different in structure from that predicted by theory (see ref. 10). Such a change in slope could be due to a breakdown of the thin disk approximation. If the disk became thick it would radiate as a rapidly rotating star¹⁰. This would satisfy the observed far-UV energy distribution (Fig. 2) and could explain the absence of evidence for a boundary layer through occultation effects and/or reprocessing of radiation.

With the outburst flux distribution for SS Cyg (Fig. 2) a bolometric luminosity can be computed. The shape of the far-UV distribution suggests that only a small amount of energy ($\approx 15\%$) is intrinsically emitted shortward of 912 Å. We find that the luminosity for SS Cyg in outburst is $1.3 \pm 0.3 \times 10^{34}$ erg s⁻¹ ($\sim 3.3 L_{\odot}$), assuming a distance of 50 pc (ref. 11). Similar arguments can be made for U Gem. However, the absence of adequate ground-based data makes the resultant luminosity very uncertain. Assuming a 3,000–10,000 Å distribution similar to that of SS Cyg, a luminosity of $1.5 \pm 0.5 \times 10^{34}$ erg s⁻¹ is derived for a distance of 75 pc (ref. 12).

As discussed earlier, the only difference between the SS Cyg and U Gem far-UV flux distributions is the presence of Lyman series hydrogen absorption in SS Cyg. [Note neither SS Cyg nor U Gem show any suggestion of He II (specifically 1,085 Å) absorption.] The identification of this line as Lyβ and not O VI is based on the results of a division of the SS Cyg distribution by that of U Gem. This division revealed additional weak absorptions at Lyγ and Lyδ. The data from the earlier outburst observed in SS Cyg with Voyager also show Lyβ absorption. Although in this outburst the line is quite variable in strength. We postulate that most of the observed hydrogen absorption in SS Cyg originates in a wind. Such winds have been observed in other dwarf novae of low inclination¹³. The absence of wind lines in U Gem is also consistent with this model. Interstellar Lyβ is also a likely contributor to the absorption in SS Cyg. However, the observed variability of the feature suggests that it cannot be the principle source of the line.

The principal results from the Voyager UVS investigation of SS Cyg and U Gem in outburst can be summarized as follows. The far-UV light curve confirms the earlier observation of a delay in the rise of flux at shorter wavelengths relative to that occurring at longer wavelengths in the early stages of an outburst. The flux distribution in the far-UV is significantly flatter ($F_{\lambda} \propto \lambda^{-0.5 \pm 0.5}$) than that in the near-UV (IUE) region. No extreme-UV emission was detected in either SS Cyg or U Gem and the shape of their far-UV flux distributions suggest that little intrinsic extreme-UV flux is emitted. A bolometric luminosity of $1.3 \pm 0.3 \times 10^{34}$ erg s⁻¹ is derived for SS Cyg in outburst ($d = 50$ pc). A similar luminosity, $1.5 \pm 0.5 \times 10^{34}$ ergs s⁻¹, is deduced for U Gem ($d = 75$ pc). No evidence is found for any significant area of the inner disk/boundary layer region with a temperature in excess of $\sim 50,000$ K. The far-UV flux distribution can be best represented by a high gravity ($\log g \sim 6-8$) intermediate temperature ($T_{\text{eff}} \sim 50,000$ K) stellar atmosphere. These results argue for a significantly different inner disk/boundary layer structure than current theory predicts. Further observations of cataclysmic variables, simultaneous with observations for IUE and ground-based telescopes, are planned.

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Upper limit on solar interior rotation

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The power spectrum of solar total irradiance (flux) variations from the Active Cavity Radiometer Irradiance Monitor (ACRIM) on the Solar Maximum Mission (SMM) spacecraft shows individual 5-min *p*-mode oscillations^{1,2} of spherical harmonic degree $l = 0-2$ and radial order $n = 16-26$. An *m*-state splitting analysis based on the widths of the (n, l) multiplets in the spectrum of ACRIM data yields a mean (sidereal) interior rotation rate between 0 and 2.2 times the observed 0.456-μHz equatorial surface rate, consistent with the rapid rotation rate originally claimed by Claverie *et al.*³ based on a (controversial) splitting interpretation of the these 5-min modes seen in line-of-sight velocity. Rotationally split *p*- and *g*- and *f*-modes have been identified in the temporal power spectrum of the limb-darkening data of Bos and Hill⁴, and from these splittings two internal rotation curves^{5,6} have been deduced which imply a solar gravitational quadrupole moment J_2 large enough to spoil the precise agreement between general relativity and observations of planetary motion. The splitting of the low- l 5-min *p*-modes implied by these curves is inconsistent with the upper limit derived here, and the reported conflict with einsteinian theory, is therefore, premature.

In this analysis I assume that the fluid angular velocity within the Sun has a radial dependence of the form $\Omega = \Omega(r)\hat{z}$. The axis $\hat{z}$ is known from surface motion. For a rotation law of this form, the frequency of a free-oscillation mode depends on the quantum numbers (nlm) of the mode as⁷:

$$\nu_{nlm} = \nu_{nl} + m\Delta_{nl} \quad (1)$$

where Δ_{nl} , defined in an inertial frame, is an integral over radius of $\Omega(r)$ times a weighting function. Explicit expressions for the weighting functions, or splitting kernels, are given in ref. 7. (Other sources of splitting, such as a magnetic field, will be ignored in this analysis.) For rotation curves which are suitably smooth functions of r , the low- l 5-min *p*-mode splitting is adequately approximated by the expression⁸

$$\Delta = \int_0^R \frac{\Omega(r)}{2\pi} \frac{dr}{c(r)} / \int_0^R \frac{dr}{c(r)} \quad (2)$$

in which $c(r)$ is the sound speed at radius r . I further assume that the modes are excited independently and isotropically, and that the mean energy per (nlm) mode depends only on frequency, in accordance with the overall distribution of power in peaks of different l as observed in the power spectrum of both the Nice data^{8,9} and the ACRIM data.

The present conclusions come from measurements of the width of several $l = 0$ and 1 peaks near the maximum of the 5-min mode excitation envelope ($\sim 3,100$ μHz). The $l = 2$ peaks were not quantitatively analysed because of their small signal-to-noise ratio. Because the axis $\hat{z}$ is nearly perpendicular to our line-of-sight, only even $l + m$ components should be visible in whole-disk measurements. I therefore assume the underlying line profile (in excess of background) of each $l = 1$ peak to be a superposition of two identical lobes, representing the $m = \pm 1$ components. The observable separation of the lobes, S , is related to the sidereal splitting Δ in equation (1) by

$$S = 2(\Delta - \nu_e) \quad (3)$$

where $\nu_e \approx 0.03$ μHz is the orbital frequency of the Earth about the Sun. The separate lobes are assumed to have lorentzian line shapes. The $l = 0$ peaks are represented by a single such lobe. Defining the linewidth as the width of a symmetrically placed bin containing half the power in the line², the width of the assumed doublet profile, W_1 , the width of the individual lobes,

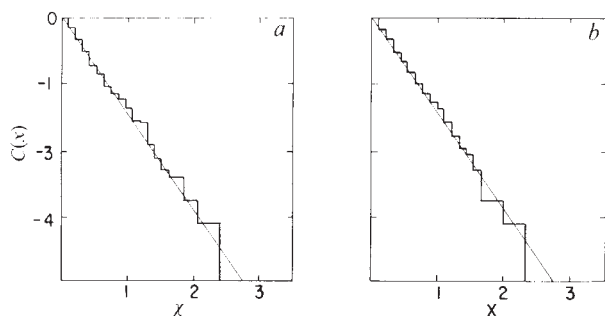


Fig. 1 *a*, The logarithm $C(x)$ of the fraction of frequency points for which the spectral power estimate p'_ν exceeds the true power by the factor x (arbitrary units), plotted for frequencies taken from 1.86- μ Hz bandwidth intervals centred on 5-min p -mode resonances in the periodogram of ACRIM data. (The true spectral power p_ν was determined from the mode parameter estimation scheme of ref. 2.) Eight intervals were selected, corresponding to the peaks $l=0$, $n=20-23$ and $l=1$, $n=19-22$. The frequency sampling of the periodogram is 29.1038 nHz so that 64 frequencies per mode were used. *b*, $C(x)$ plotted from 512 frequencies belonging to a background interval in the 5-min range. The linearity of these plots corresponds to a χ^2 distribution with 2 degrees of freedom, the distribution assumed in the error analysis. (This method of display was suggested by P. Delache.)

W_0 , and the separation, S , obey a pythagorean relationship:

$$W_1^2 = W_0^2 + S^2 \quad (4)$$

The differences between the shapes of 5-min peaks in the ACRIM data and the idealized line profiles discussed above are assumed to be mainly statistical: thus linewidth measurements are subject to random error. Accordingly, I assume that the spectral power estimates p'_ν at frequencies separated by more 0.04 μ Hz (the resolution of 290 days of data) are uncorrelated, and that p'_ν at each frequency ν comes from a χ^2 distribution with 2 degrees of freedom, a distribution typical of a periodogram of filtered noise. The mean of the χ^2 distribution, p_ν (the true spectral power that p'_ν estimates), presumed to be a superposition of idealized 5-min mode profiles and a nearly flat background 'continuum', was estimated from the parameters of the individual 5-min modes² and the observed continuum.

Two tests demonstrate the credibility of these statistics. To check that the periodogram conforms to the stated distribution I have plotted $C(x)$ against x in Fig. 1, where $\exp\{C(x)\}$ is the fraction of periodogram points for which p'_ν/p_ν exceeds x . The straight line plots confirm the expected $C(x) \propto -x$ dependence, both for frequency intervals containing 5-min modes and for background intervals. To verify the statistical independence of the spectral estimate at frequencies differing by more than 0.04 μ Hz, I smoothed the periodogram and measured the decrease in the scatter of the points. The ratio of the variance in the smoothed periodogram to that in the original should equal 0.04 μ Hz divided by the effective resolution of the smoothed periodogram. This dependence was confirmed for frequency ranges of interest.

The operational definitions of power and linewidth² systematically underestimate the true power and width because the power in the modes is integrated only over a 5- μ Hz interval. A Monte Carlo simulation was performed to calibrate empirically the relation between the true power and width of the $l=0$ modes and the expected value of the operationally defined power and width, and to determine the random error of the measurement. In this, and in subsequent simulations, a statistical ensemble of artificial periodograms containing modes and background were generated with the statistical properties assumed for the real data. Ignoring the possible variation of linewidth with frequency, the true width inferred for the four strongest $l=0$ lines ($n=20-23$) is $\Delta\nu = 1.6 \pm 0.3$ μ Hz.

A second Monte Carlo calculation calibrated the relation between the width W_1 and power of the $l=1$ doublets and the operationally-defined linewidth and power, assuming that the

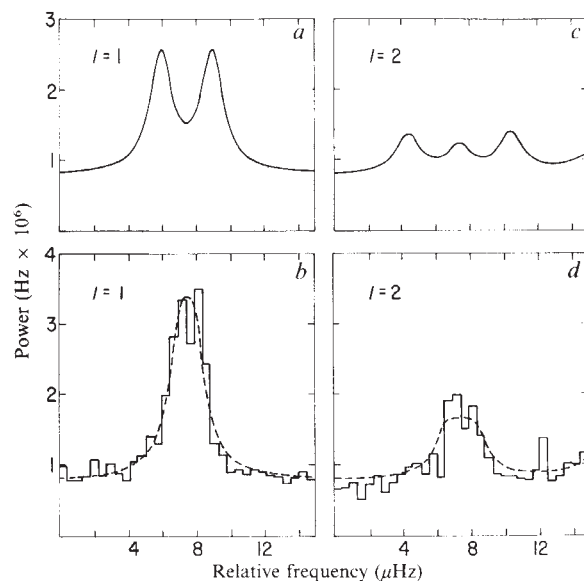


Fig. 2 *a*, Theoretical $l=1$ profile with a lobe separation of 3.0 μ Hz, that is a splitting $\Delta = 1.56$ μ Hz as defined in the text, slightly less than the separation implied by the rotation curves of refs 5 and 6. The total power in the doublet equals the mean power of the $l=1$ modes studied in this analysis and the width of the lobes equals the estimated 1.6- μ Hz width of the $l=0$ modes in the same frequency range. The continuum power level is that of the ACRIM data. The theoretical line shape corresponding to the larger splitting stands in marked contrast to the appearance of the actual modes. *b*, Superposition of three $l=1$ peaks ($n=20-23$) in the ACRIM periodogram, based on the measured frequency centroids, plotted at a resolution of 0.4657 μ Hz. The smooth curve is a doublet with a (synodic) lobe separation $S = 0.86$ μ Hz, the separation expected from solid-body rotation with a ~ 25 -day sidereal period and represents the theoretical appearance of a smoothed periodogram based on an unlimited amount of data. *c*, Idealized $l=2$ profile resulting from the $\Delta = 1.56$ - μ Hz splitting is also at variance with the data and further substantiates our conclusions. The relative power 3:2:3 in the $m=-2$, 0, and $+2$ lobes shown and the absence of the $m=\pm 1$ lobes in full-solar-disk measurements is dictated by isotropy and by the assumption of a solar rotation axis perpendicular to our line of sight. *d*, The superposition of the four $l=2$ modes ($n=19-22$) is also consistent solid-body rotation (the smooth curve again indicates the theoretical profile which best fits the data).

doublet components have the same width as the $l=0$ modes in the same frequency range (that is, $W_0 = \Delta\nu$). This gives a mean width for the three $l=1$ modes ($n=20-22$) of $W_1 = 1.9 \pm 0.2$ μ Hz. A composite of the three observed $l=1$ modes appears in Fig. 2. The value of Δ inferred from W_0 and W_1 through equations (3) and (4) is $0.5 (+0.2, -0.5)$ μ Hz, consistent with the 0.456- μ Hz splitting expected from solid-body rotation with a ~ 25 -day sidereal period, but also consistent with any rotational rate between 0 and 1.4 times the surface rate (1σ error limits). Note that only the magnitude of S (in equation (3)) can be determined from the (full-disk) ACRIM data. An unambiguous value for Δ was obtained by assuming that S is positive, in accordance with a net internal rotation having the same sense as the surface rotation.

Relaxing the assumption $W_0 = \Delta\nu$, made above, leads to a more rigorous upper bound on splitting. The probability of obtaining an $l=1$ linewidth smaller than the observed one, for various hypothetical values of Δ , was computed from a Monte Carlo program. In this final program, the influence of oscillations aliased into the 2,900–3,300- μ Hz range by the ACRIM shutter operation² was also simulated. Based on the published analysis of the Nice group¹⁰, whose velocity oscillation spectrum extends well beyond the 3,814.7- μ Hz ACRIM Nyquist limit, the spectral power per multiplet of given l is at least three times greater at the frequencies of interest than at the frequencies of multiplets that would produce aliases. The m -state width of the potentially aliased multiplets is assumed to be 5 μ Hz. For each point on a

grid of mode parameters (Δ , W_0 , and parameters defining the power and frequency of the aliases) a number of trial measurements were performed. A trial consists of generating three independent periodograms based on the same profile, measuring the doublet width in each, and averaging the three measurements. For $\Delta = 1.0$ and $2.0 \mu\text{Hz}$, 256 and 1,024 trials were made, respectively, for each value of the other parameters. (The ranges of the other parameters reflect their uncertainty, in particular W_0 was assumed to lie in the range 0.6 – $1.8 \mu\text{Hz}$.) At the lower splitting only one trial (at most) per grid point resulted in a mean width smaller than the observed one, and at the larger splitting there were no such occurrences. I thus infer a strong ($\approx 2.5\sigma$) upper limit $\Delta_{\text{max}} = 1.0 \mu\text{Hz}$ on the rotational splitting.

These results demand comparison with internal rotation curves obtained from different analyses of rotationally split modes in the solar limb-darkening data⁴. Hill *et al.*⁵ have derived a rotation curve from these data which implies a solar mass quadrupole moment $J_2 = (5.5 \pm 1.3) \times 10^{-6}$, in conflict with the einsteinian general relativistic interpretation of Mercury's perihelion motion at the 2σ level. From a preliminary analysis of

the same data, Gough⁷ obtained $J_2 = 3.6 \times 10^{-6}$, not large enough to conflict with general relativity. Both analyses used seven multiplets for the curve fitting, a common set with the exception of two p -modes. A more recent analysis by Campbell *et al.*⁶ essentially confirms the Hill *et al.* rotation curve, giving $J_2 = 5.0 \times 10^{-6}$, again in conflict with relativistic predictions.

To test the conclusions derived from the limb-darkening data I have calculated, using equation (2), the splitting of the low- l 5-min modes implied by the curves used to derive the quoted J_2 values. The function $c(r)$ was taken from a recent standard solar model calculation¹¹. The rotation curves in refs 7, 5, and 6 imply $\Delta = 1.46$, 1.70 , and $1.69 \mu\text{Hz}$, respectively, in conflict with the upper limit $\Delta_{\text{max}} = 1.0 \mu\text{Hz}$ derived here. Thus the basis established in refs 5 and 6 for conflict between general relativity and solar oscillation data is removed by the SMM data.

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A new imaging proportional counter using a Penning gas improves energy resolution

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In recent years Siegmund *et al.*^{1,2} have attempted to combine in one detector the position resolution of the multi-wire proportional counter (MWPC)^{3–5} or the parallel-plate imaging proportional counter (PIPC)^{6,7} with the energy resolution of the gas-scintillation proportional counter (GSPC)^{8–11}, using light from avalanches in a PIPIC. By counting the light flashes from individual primary electrons, Fano factor limited energy resolution was obtained but only for energies below 2 keV. The position resolution was $\approx 350 \mu\text{m}$ over an active diameter of 25 mm. Sipilä and co-workers^{12,13} used Penning gas^{14,15} mixtures to improve the energy resolution to 11% at 6 keV. However, this was achieved at gas gains of <50 , and in a single wire proportional counter (PC) without imaging capability. We have achieved similar resolution with gains up to 550 by using a Penning gas in a uniform field. Although satisfactory for energy measurement down to 0.1 keV this gain is not high enough for good position resolution in a PIPIC. We therefore use another stage of uniform field avalanche to boost the overall gain to values $>10^5$. The concept of two-stage avalanching was first reported by Chrapak *et al.*¹⁶ and used by Breskin *et al.*¹⁷. We have built and tested a laboratory version of such a detector (Fig. 1), which we call the Penning gas imager (PGI). A Penning gas and a two-stage parallel grid avalanche geometry are combined with a wedge and strip anode (WSA) position read-out system¹⁸. Preliminary experimental energy resolution is 12% FWHM at 6 keV (24% FWHM at 1.5 keV) and the position resolution is 100 μm FWHM at 1.5 keV. This compares with $\approx 10\%$ and $\approx 1 \text{ mm}$ for the GSPC and $\approx 18\%$ and a few hundred micrometres for the MWPC. The particle background can be reduced by an estimated 99% using an anticoincidence technique.

The energy resolution of a PC is limited by both Fano and avalanche statistics. The FWHM resolution is given by

$$R_E(\%) = 236[w(F + f + g)/E]^{0.5}$$

where w is the mean energy to produce one ion pair, F the Fano factor, f the avalanche factor, g a broadening factor depending on the small scale grid or wire geometry, and E is the X-ray energy.

The value of F for argon (and conventional PC gas mixtures such as Ar/CH₄) is 0.17 and f is typically 0.65 (ref. 19). With $w = 26.2 \text{ eV}$ in argon this gives $R_E = 35E^{-1/2}$ where E is in keV. This assumes $g = 0$. In practice this resolution is not achieved due to irregularities in the anode wires²⁰ or the grids⁷; 18% at 6 keV is a typical value and this implies a g factor equivalent to 10% extra broadening.

In the GSPC the f and g factors are eliminated by not using avalanches. In principle, $R_E = 16E^{-1/2}$ can be achieved but a typical observed value at 6 keV is 10% compared with a theoretically possible 6%. This corresponds to an additional broadening of about 8% due to gas impurities and geometry dependent light losses in the counter.

The energy resolution of the PGI is potentially as good as that of the GSPC. By using argon with the Penning admixture C₂H₂ which has an ionization potential lower than the energy of the photons emitted by the excited Ar atoms, the admixture can be photo-ionized. Also metastable argon atoms can collisionally ionize the ethyne molecules. This process, known as the Penning effect, produces a dramatic increase in α , the first Townsend coefficient¹⁵. As an example, the changes in the relative efficiencies of the various processes such as excitation and ionization in a Ne/Ar mixture are shown in Fig. 2. In the initial ionization, more of the available energy of the primary photo-electron and Auger electron is used for ionization (the value of w is reduced from 26.2 to 20.3 eV)¹⁹ and in addition the fluctuations in the energy losses are smaller. The Fano factor is thus reduced¹⁹.

In the avalanche, using the model of Alkhazov¹⁹ and the data on α of Kruithof and Penning, Sipilä¹³ shows that for an optimum value of the field strength, f approaches zero in a (Penning) mixture of Ne and Ar. As the process for Ar/C₂H₂ is similar such a result can also be expected for this mixture. A theoretical energy resolution of $10E^{-1/2}$ is then available. Sipilä's results were obtained at low gas gains (<50) but the resolution in conventional PCs rapidly degrades with increasing gain. This is due to the strongly varying field near the anode wire which makes it impossible to use the optimum field^{15,19} needed for low values of f . Sipilä was aware of this limitation and suggested the use of a uniform field avalanche¹³. In the PGI the parallel geometry provides a uniform field and much higher gains can

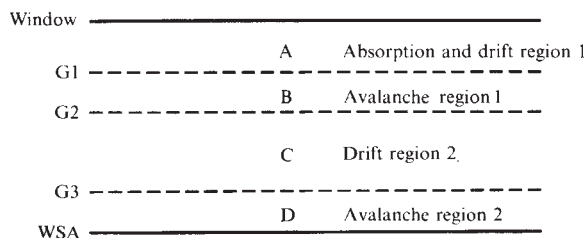


Fig. 1 Schematic diagram of the PGI. X rays enter through the thin polypropylene window and are absorbed in region A. Electrons from the resulting ionization cloud drift in a weak field through grid G1 and avalanche in the higher field of region B. The energy signal is taken from G1 and/or G2. About 20% of the avalanche electrons are effectively transferred to the second drift region C where the cloud is allowed to diffuse before reaching the second avalanche region D. The position of the electron cloud's centroid is determined using the signals from the WSA.

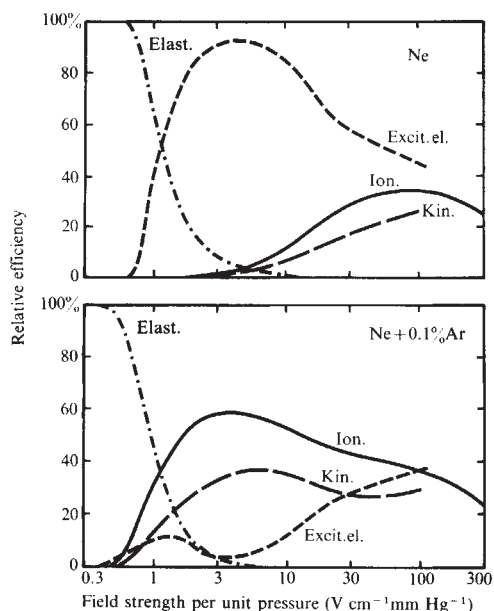


Fig. 2 Fraction of the total energy received by the electrons from the electric field which is spent in elastic collisions (Elast.), excitation of electronic levels (Excit. el.), excitation of vibrational levels (Excit. vib.), ionization (Ion.) and increase of kinetic energy of the electrons (Kin.). (From ref. 26.)

be used without the accompanying increase in R_F . With a gain of 550 we have obtained 12% FWHM at 6 keV and 24% at 1.5 keV in the laboratory detector. These measurements were made using argon with 0.5% C_2H_2 . The energy resolution as a function of X-ray energy is plotted in Fig. 3. With a g factor providing a 10% broadening (we have measured 18% at 6 keV in the PGI using Ar/10% CH_4) we expect that optimizing the grid geometry would improve the energy resolution to <10% FWHM.

The gain of the first avalanche stage is set at ≈ 500 , and $\approx 20\%$ of the electrons produced here are transferred through grids G2 and G3 to the second avalanche region D. Due to the Penning effect, UV photons which pass through G2 ionize admixture molecules in region C. This process is independent of any applied field. In contrast, electron transfer in conventional counter gases can at best only take place in the ratio of the field strengths below and above the grid²¹. It has been shown²² that with their multi-step chamber Breskin *et al.* required a Penning gas to obtain transfer at all. This is because their grids were composed of thin wires onto which the avalanches took place (not in a true parallel field) and this has the effect of reducing the transfer in a Penning gas to that of a conventional gas.

The second stage of amplification is used to boost the overall gain to $>10^5$. This is necessary for the WSA position read-out

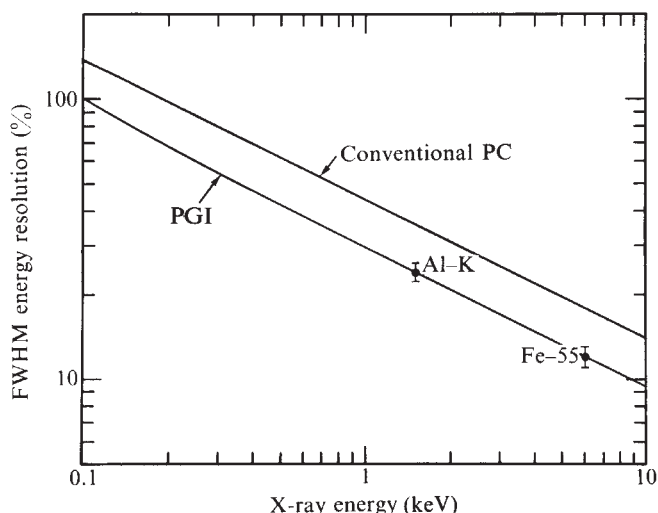


Fig. 3 The energy resolution of the PGI as a function of X-ray energy. Indicated are the resolution curves for a conventional proportional counter (PC) and for the PGI. The slight upturn in the PGI curve is due to the electronic noise contribution.

system which consists of three conductive electrodes deposited onto an insulating substrate. Their geometry is such that a linear, two-dimensional positional variation in charge output is obtained, allowing the position of an event to be determined by a simple algorithm. Preamplifier noise is associated with this charge measurement. In addition the division of charge among the discrete electrodes is binomially distributed and this gives rise to a contribution to the position resolution called partition noise.

In the same laboratory detector (70 mm diameter) we obtain a position resolution of 100 μm FWHM at 1.5 keV with a gain of 1.3×10^5 . We have also built a PGI with a 200 mm diameter WSA whose overall noise is such that for all but the lowest energies (<0.5 keV) the resolution is dominated by electron diffusion in the absorption region (A) while at the higher energies the effect of primary electron track length²³ is dominant. This represents >1,000 FWHM resolution elements over most of the energy range.

In space, the background count rate due to charged particles is a problem for X-ray astronomy. This background is most effectively reduced^{24,25} by surrounding the X-ray detection volume except for the entrance window by anticoincidence guard detectors. In the case of the PGI we are able to surround the WSA by an annular anode and use it with the second drift/avalanche region to provide such a detection volume. By comparison with previously flown detectors using this type of background rejection scheme, we expect to be able to reject >99% of the particle events in space^{24,25}.

Finally, several further improvements to the PGI are under consideration. First, the use of a xenon based Penning mixture as the filling gas will increase the quantum efficiency (QE) of the detector at high energies (in argon the QE is typically 70% at 1.5 keV and 35% at 6 keV). Second, a fast high-voltage switching circuit can be used to make the number of electrons reaching the WSA independent of the X-ray energy. This feedback system reduces the dynamic range needed for position signal processing by a factor of about 80. Third, cooling of the first stage preamplifier would improve the energy resolution at low X-ray energies by reducing the contribution due to electronic noise.

We are designing a flight version of the PGI which, with an imaging X-ray telescope will form the payload of an Aries sounding rocket to be launched in 1985.

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Effect of a heated patch of auroral ionosphere on VLF-radio wave propagation

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In the early 1960s, during the period of atmospheric nuclear tests, much theoretical interest developed in the effects of localized ionospheric depressions on the propagation of very low frequency (VLF) radio waves^{1–4}. Similar VLF-propagation effects are also produced by the localized dumping of electrons from the radiation belts after wave-particle interactions^{5,6}. Both nuclear explosions and particle precipitation events are of a transient nature, however, and no experimental study has yet been made to confirm these early theoretical predictions. With the development of a unique high frequency (HF) heating facility near Tromsø, Norway, the generation of movable controlled anomalies in the D-region has become possible. We describe here some initial observations, made in Norway, of the effect of such a movable D-region anomaly on the VLF signals received from the 12.1-kHz Omega transmitter at Aldra. The observations confirm the validity of earlier theoretical predictions.

Figure 1 shows the region of northern Scandinavia pertinent to the experiment. The VLF receiver used for the measurements was located at the Skibotn Astronomical Observatory, 443 km north-east of the Omega VLF transmitter at Aldra. The HF heating facility was located at Ramfjord, 15 km south of Tromsø.

The heater antenna array consisted of six rows of six crossed full-wave dipoles⁷. By suitably adjusting the relative phases of the drives to the different rows of the heating antennas, it was possible to deflect the heating beam $\pm 56^\circ$ from the vertical in the north-south plane⁸. Assuming a height of 80 km for the region of maximum D-region heating⁹, the volume of maximum heating effect, defined by the -3 dB points, could thus be displaced 120 km north or south of Ramfjord. However, while the heated region was circular with ~ 20 -km diameter when the beam was vertical, the heated volume had an elliptical cross-section with major axis of ~ 70 km and minor axis of ~ 38 km (see Fig. 1) when the beam was displaced 56° south or north. The experiment reported here simply entailed observing the phase and amplitude of the normal magnetic component of the

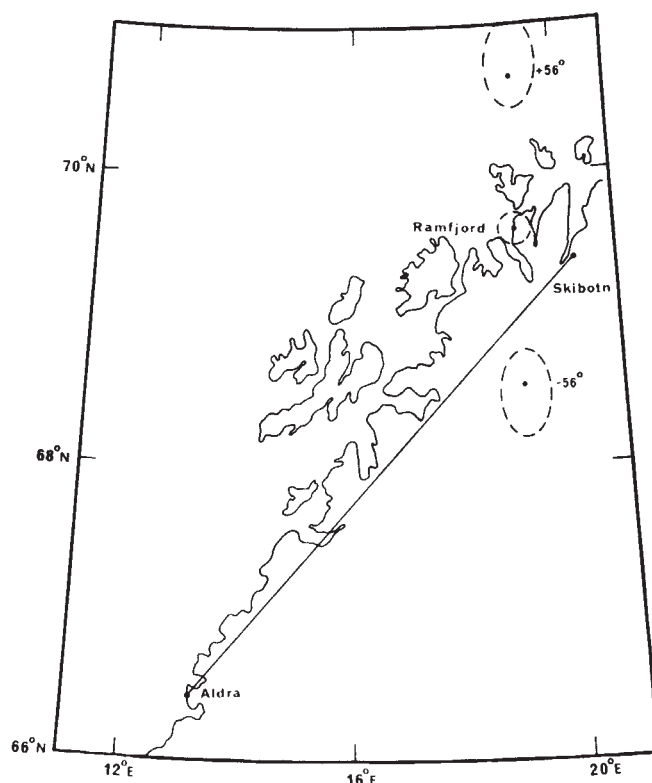


Fig. 1 Map of northern Scandinavia showing the location of the Omega station at Aldra, the VLF receiving station at Skibotn and the heating facility at Ramfjord. The dashed circle and dashed ellipses are approximate representations of the antenna -3 dB (half power) points at 80 km. The circle applies to a vertical beam, the ellipses to a beam deflection of 56° from the vertical to the north or south of the transmitter.

12.1-kHz signals from the transmitter as the heater beam was deflected across the Skibotn/Aldra propagation path. Due to the fixed locations of the heating facility and VLF transmitter, it was not possible to locate a land-based VLF receiver at a sufficiently high latitude that the heater modified the midpoint of the VLF propagation path. The optimum configuration adopted by Jones *et al.*¹⁰ was thus not possible. It was therefore decided to locate the receiver at the most convenient experimental location, this being the Skibotn Astronomical Observatory. With this geometry it was decided that the total normal magnetic component of the VLF signal should be recorded with the eventual aim of analysing the data using a full waveguide analysis based on the work of Crombie⁴ and Wait³, the simpler analysis in terms of reflection coefficients not being feasible with such an asymmetric geometry.

Calculations⁹ show that for an effective radiated power (ERP) of 300 MW at 2.759 MHz in the x-mode, the collisional frequency in the D-region increases by at least a factor of 5 over a height range of ~ 10 km. Assuming that this modification can be modelled as a 10-km elevation of the ionosphere over an area of 30 km diameter: this increased collisional frequency results in an increase of the effective VLF reflection height by 10 km, then according to the work of Field and Engel¹¹ a wave passing through the midpoint of such an elevated region would suffer a phase change $\sim -0.4^\circ$. Experimental verification of such a small effect on VLF propagation thus entails measurement of VLF phases angles with a resolution of better than $1/10^\circ$.

Resolution is not the only significant parameter in the experiment, however, the phase stability during the experiment is also important. Experience suggests that long-term stability of VLF propagation in the auroral zone is not high^{12,13} and thus the technique described below was used to offset the effect of ionospheric variability on a time scale of > 2 s.

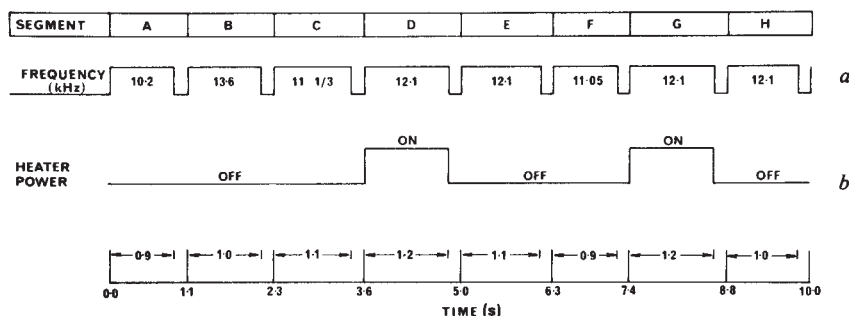


Fig. 2 a, Norwegian Omega transmitter format. b, Heating transmitter switching format. Cycles repeat every 10 s.

Figure 2a shows the Omega transmission format, together with the four periods of 12.1-kHz transmission. Figure 2b shows the period during which the heater was in operation (2.759 MHz, ERP = 200 MW). As can be seen, the heater was on for the first and third 12.1-kHz segments. By subtracting phase measurements recorded between the first and second or third and fourth 12.1-kHz segments the effect of heating could be determined over a 2-s interval, thus reducing the effect of longer-term phase variations. By operating the heater with this pulse format for 1 min, at each setting of a beam deflection angle, it was thus possible to get a mean of 12 phase difference readings at each position of the beam.

Figure 3 shows the phase and amplitude anomalies on the normal magnetic component of the Omega 12.1-kHz signal at Skibotn, recorded using the above technique. The readings were obtained from 13.45 to 14.24 UT on 29 September 1983 whilst the beam was deflected from 16°N of Ramfjord to 56°S. Although the maximum phase anomaly is quite small, typically $\pm 0.5^\circ$, there is obviously a clear oscillatory variation of phase with position of the beam as predicted by the earlier theoretical works²⁻⁴. The maximum oscillations in amplitude are also very small, only 0.1 dB, and yet they also clearly show the same oscillatory behaviour with maximum phase displacements occurring at the same deflection angle as minimum amplitude displacements and vice versa. This 90° phase shift in amplitude and phase oscillations is in excellent agreement with the work of Wait³ displayed in Fig. 4. The dashed and solid curves show the amplitude and phase oscillations computed for an anomaly 100 km square as it moves along the perpendicular bisector of a 10^7 -m propagation path. The coincidences of maxima in phase deflection with minima in amplitude are clearly evident. (The amplitude and phase anomaly scales are normalized to those produced by a similar depression extending the full width of the path.) The experimental results can be seen to be in excellent qualitative agreement with the theory. The quantitative agreement between theory and experiment will now be considered.

If the effect of the heater can be modelled as a 10-km elevation of the ionosphere over a circular area of 30 km diameter, then the theory of Field and Engel¹¹ and Wait¹ predicts a phase anomaly of -0.4° for a ray passing through the middle of the elevated region. The heated patch in this experiment is approximately centred on the VLF propagation path for a beam deflection angle of 40°S (see Fig. 1). At this position the patch is elliptical with major and minor axes of ~ 36 and ~ 28 km respectively. The phase perturbation of -0.42° observed at this angle (see Fig. 3) is thus in excellent agreement with theoretical predictions.

The early theoretical work of Crombie⁴ and Wait¹⁻³ thus seems adequate to explain the phase and amplitude anomalies caused by ionospheric elevations and depressions both on and adjacent to VLF propagation paths.

The magnitude of the phase and amplitude disturbances observed here are of a similar order although somewhat smaller than those observed by Jones *et al.*¹⁰ using the 50-MW heating transmitter at Platteville. However, they observed the phase and amplitude anomalies on the 'abnormal' magnetic component received on a 128-km propagation path when only the midpoint of the path was heated by a fixed HF beam. The total normal magnetic field was recorded in this study, which inevitably

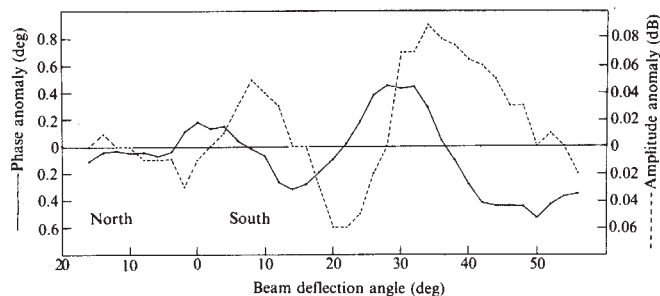


Fig. 3 Experimentally-determined values of phase and amplitude anomalies. The anomalies are plotted as a function of angular deflection of the heating beam from the vertical.

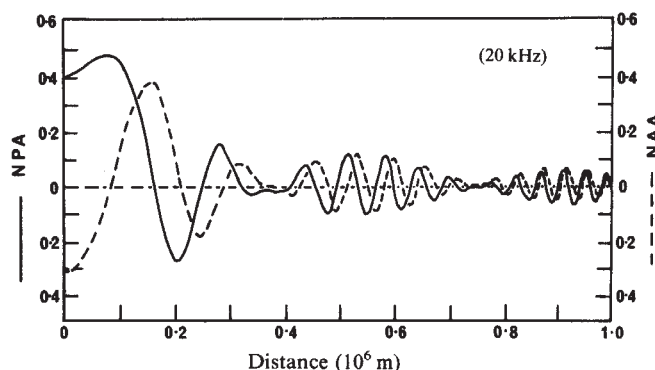


Fig. 4 Theoretical estimates of the normalized phase anomaly (NPA) and normalized amplitude anomaly (NAA) produced by an ionospheric depression 100 km square. The normalized values are plotted as a function of distance of the depression along the perpendicular bisector of the transmitter-receiver propagation path, measured from the midpoint of the path (after ref. 3).

included a large component of unmodified ground-wave and this may account, to some extent, for the smaller effects observed in this experiment.

Note that the work of Wait¹⁻³ and Crombie⁴ applies only to single-mode propagation, whereas on the short paths used in this experiment a number of modes probably contribute to the received signal even under daylight ionospheres. In nighttime conditions multimode propagation will almost certainly be significant and enhanced anomalies due to heating may be expected⁶. Similar enhancements, but invoking multipath propagation within the ionosphere, have been used to explain large amplitude anomalies on steep incidence LF data¹⁴. Since this initial study, further experimental observations have now been made in both daytime and nighttime conditions, and enhanced effects due to modal interference have been observed. These results will soon be submitted for publication, together with a full wave analysis of the diffraction effects produced by a heated patch of ionosphere in multimode propagation conditions.

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Delayed phase change due to hot asthenosphere causes Transantarctic uplift?

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The Transantarctic Mountains are one of the world's major mountain chains, extending 3,500 km across Antarctica in a belt 200–500 km wide (Fig. 1). The region has been uplifted by at least 4 km since late Mesozoic time, yet there is none of the thrusting, folding, regional metamorphism and andesitic volcanism associated with many other large Cenozoic mountain ranges and apparently related to subduction and subsequent continental collision. Instead, the uplift of the Transantarctic Mountains is attributed here to the delayed effects of the overriding by east Antarctica of anomalously hot asthenosphere forming under west Antarctica in the late Cretaceous. Temperature increases of 100 °C are estimated at the base of the lithosphere, with mantle heat flux increasing by $\sim 3.5 \text{ mW m}^{-2}$. These changes cause an inferred uplift rate of 90 m Myr⁻¹ some 50 Myr later. The late Cenozoic volcanism in the Transantarctic Mountains, typified by activity in Victoria Land, is attributed to the previously heated continental lithosphere overriding hot asthenosphere that was brought under Antarctica when it separated from Australia.

The continental uplift model used here proposes that rapid uplift will lag some 50 Myr behind the overriding of anomalously hot asthenosphere by a continent. An estimated mantle heat flux increase of 3.5 mW m^{-2} is believed to cause phase changes in the uppermost continental mantle¹. In the absence of palaeo-flow models for the asthenosphere, empirical reference frames must be used to locate hot asthenosphere and relate it to plate motions.

For interpreting the later Cenozoic uplift of southern Africa, a reference frame fixed to South America seems to be useful¹. It has been suggested that anomalously hot asthenosphere in this frame was located at the positions of former oceanic ridges. It has also been assumed that when continental lithosphere moves over former ridge positions in this frame the hot oceanic asthenosphere is overridden by the continent rather than being carried with the oceanic lithosphere. Such a continental area would experience uplift and a higher mantle heat flux which would affect the postulated phase changes near the Moho some 50 Myr later. Later Cenozoic uplift of south-east Australia appeared to be accounted for by this model. The 'hotspots'

reference frame apparently did not account for the uplifted regions of southern Africa. This frame is probably as good as the South American frame for discussing the uplift of south-east Australia, although a comparison was not made in the original work. In the case of the Antarctic, the hotspots frame of Morgan² gives similar results, largely because South America has not moved much relative to the hotspots frame in south polar latitudes.

Why these particular frames are useful is not well understood. They may reflect the fact that zones of upwelling asthenosphere move slowly in a fixed lithospheric angular momentum reference frame^{3–5} and that South America approximates to such a frame today⁶. Their usefulness supports the assumption that hot oceanic asthenosphere can be overridden by continental plates.

Late Cretaceous to early Tertiary extensional zones have been postulated in Antarctica^{7–11} to account for the development of the continental mosaic that now makes up west Antarctica. The isostatically adjusted bedrock surface of west Antarctica lies mostly at $\sim 500 \text{ m}$ below sea level, in marked contrast to the 1,000 m or more average elevation of east Antarctica¹². The presence of significant volumes of volcanic rocks at depths as great as 2 km below sea level in the Byrd sub-glacial basin, suggested by seismic¹³ and magnetic studies^{10,11}, may relate to a period of incipient ocean floor formation.

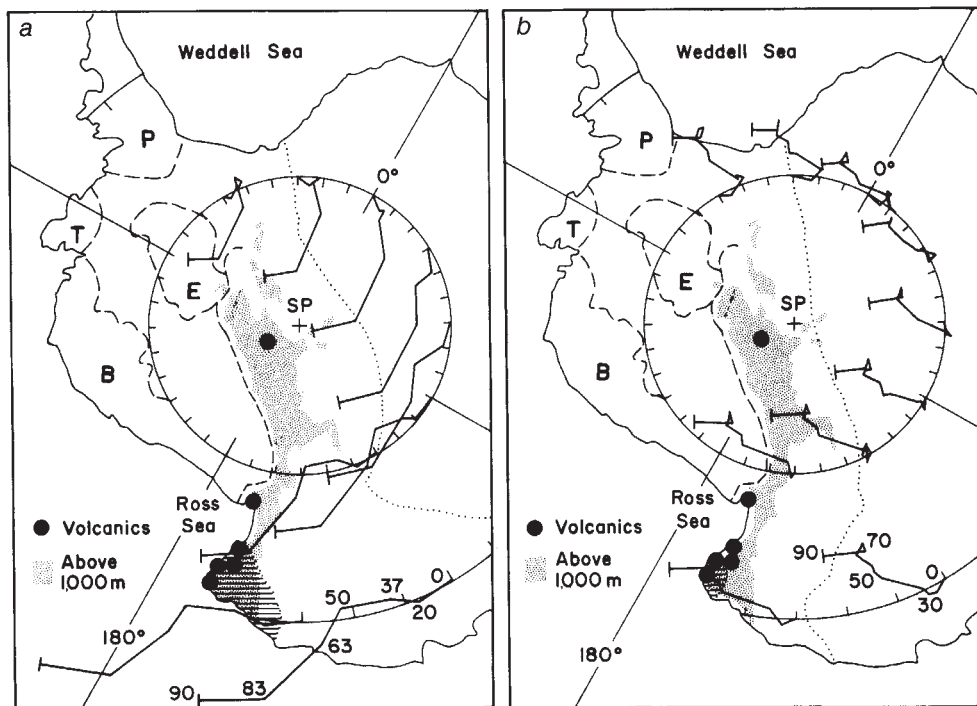
Whether or not any ocean floor was created, west Antarctica seems to have been stretched¹⁴. Such stretching would have brought hot asthenosphere nearer the surface. The age of the extensional zone, running along the edge of the Transantarctic Mountains is not known. It is probably no older than the separation of the Pacific plate from the Antarctic plate, whose age can be inferred to be about 91 Myr by extrapolating to closure the positions of the two plates at anomalies 29 and 32 yr (ref. 15). It is older than the anomalies now lying against the Antarctic continental edge, probably about 65-Myr old¹⁶.

Figure 1a, b show the maximum size of the area in the hot-spots and South American reference frames previously occupied by west Antarctica that could have been overridden

Table 1 Uplift rates in the Transantarctic Mountains

Age	Locality	Observation and inference
Late Pliocene (2.5–3.4 Myr)	Wright Valley, South Victoria Land (77.5°S, 162°E)	Benthic foraminifera, originally at 10–100 m below sea level, now at 160 m (ref. 24) Uplift rate = 50–105 m Myr ⁻¹
Late Miocene– early Pliocene,	Taylor Valley, South Victoria Land (77.7°S, 163°E)	Benthic foraminifera, originally 600 m now at 120 m below sea level ²⁵ . Uplift rate in past 5 Myr of 80–100 Myr ⁻¹
Miocene (15 Myr)	Miller Range, Nimrod Glacier (83.3°S, 157°E)	Glacial erosion cut at 1– 1.5 km now at 2.5 km (ref. 26) Uplift rate = 80–100 m Myr ⁻¹
15.7–18.3 Myr	Queen Maud Mountains (87°S, 154°E)	900 m of pre-volcanic relief; hence minimum height of region now at 2.5 km (ref. 27) Maximum uplift rate = 90 m Myr ⁻¹
Post-late Cretaceous	Queen Maud Mountains (86.5°S, 150°E)	'Several thousand metres' vertical movement ²⁸
>25 Myr	Transantarctic Mountains	Elevation 500–1,500 m for first highland glaciers at 25 Myr (ref. 29) Uplift rate = 40–80 m Myr ⁻¹

Fig. 1 The approximate outline of the Antarctic coast or coastal ice; a present-day geographical grid with latitude circles at 10° and longitudes at 10° ; areas of the Transantarctic Mountains higher than 1,000 m after upward correction for the present-day ice load¹² in stipple; principal late Cenozoic volcanic fields in the Transantarctic Mountains as circles; and possible boundaries between major continental fragments as dashed lines. *a*, Solid lines show the successive past positions of points on the present-day geographical grid in a reference frame fixed to South America. Only one track has been labelled, the numbers are ages in Myr back to 90 Myr. *b*, As *a* but the tracks are taken from the hot-spots frame of Morgan². The dotted line in *a* and *b* shows the present position of a line that originally lay along the boundary between east Antarctica and the western edge of the Transantarctic Mountains in each reference frame. In both cases the high topography of the Transantarctic Mountains is parallel to this boundary but well within it. The horizontally shaded area in *a* and *b* is the area in each reference frame that could have overridden a former ridge position formed in early Cenozoic time.



by east Antarctica and hence subject to later uplift in the past 90 Myr. According to the model, these areas were underlain by anomalously hot asthenosphere. The uplifted area should be smaller—because of the time lag between overriding and uplift; it should form an asymmetrical mountain range decreasing in height towards the interior of east Antarctica; and significant uplift may have started within the Cenozoic, depending on the age of the extensional zone and the time-lag between overriding and uplift. The most likely age is thus about 40 Myr. As shown below, the heat needed for the phase change is small and causes negligible thermal expansion. Thus the uplift of the Transantarctic Mountains should start at a slow rate as soon as overriding begins, but will rapidly increase once the heat reaches the phase change.

Figure 1*a, b* show the high topography of the Transantarctic Mountains allowing for isostatic uplift following removal of the present-day ice load¹². Several independent lines of evidence, summarized in Table 1, allow an uplift curve to be constructed for these mountains (Fig. 3).

Rates of uplift inferred from glaciological and palaeontological evidence (Table 1) are in reasonable agreement with 'erosion rates' deduced independently from fission track results on apatites in dated granite plutons from south Victoria Land¹⁷. They indicate slow uplift (or 'erosion rate') of 15 m Myr^{-1} for the period $\sim 157\text{--}45 \text{ Myr}$. A pronounced break was detected at 45 Myr with a subsequent rate of $\sim 93 \text{ Myr}^{-1}$ (taking an elevation of the 100°C isotherm at 3.8 km below sea level, based on an average geothermal gradient of $30^\circ \text{C km}^{-1}$ (refs 18–20)).

Some areas of plateau uplift are also associated with volcanism, but its timing and distribution are variable and therefore less easy to predict. In southern Africa there is no significant volcanism associated with Cenozoic uplift. By contrast, south-east Australia shows two distinct episodes of basaltic volcanism. The younger phase shows a southward migration attributed to the overriding of the hot asthenosphere originally under the Coral Sea¹. The time-lag between overriding and the peak of the volcanism here is $\sim 10 \text{ Myr}$.

The model suggests that if volcanism takes place it will be predominantly basaltic, although volcanism is not an *a priori*

requirement of the model. The most probable position for volcanism is within the Transantarctic Mountains, with an age of perhaps 45–70 Myr. Later volcanism might also occur in north Victoria Land, where the previously heated lithosphere of east Antarctica has overridden hot asthenosphere brought to the base of the lithosphere by Cenozoic ocean-floor spreading at the edge of the Antarctic continent. This volcanism would have started about 20 Myr ago and should be increasing in intensity, reaching its maximum development today. The predicted extent of the area affected is shown on Fig. 1*a, b*.

Basaltic volcanism in Antarctica is concentrated in several provinces, although no attempt is made here to investigate it outside the Transantarctic Mountains. Here basaltic volcanism is sited principally in Victoria Land, with a minor centre in the Queen Maud Mountains (Fig. 1*a, b*)²¹.

There is reasonable agreement between the predicted and observed timing, form and distribution of the uplift of the Transantarctic mountains. The uplift of the Transantarctic Mountains may well have been an important factor in establishing a continental ice sheet on the Antarctic. Because stages in

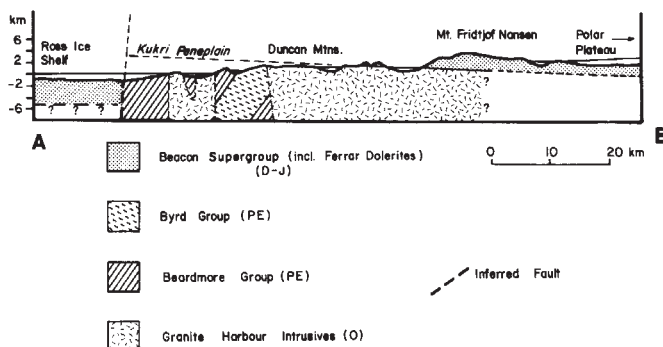


Fig. 2 Schematic cross-section through the Transantarctic Mountains at Mt Nansen (85°S , 165°W). The Kukri Peneplain separates the Beacon Supergroup (including the Ferrar dolerites) from the deformed or intrusive basement rocks. The Beacon section dips gently inland at a few degrees, from ref. 30.

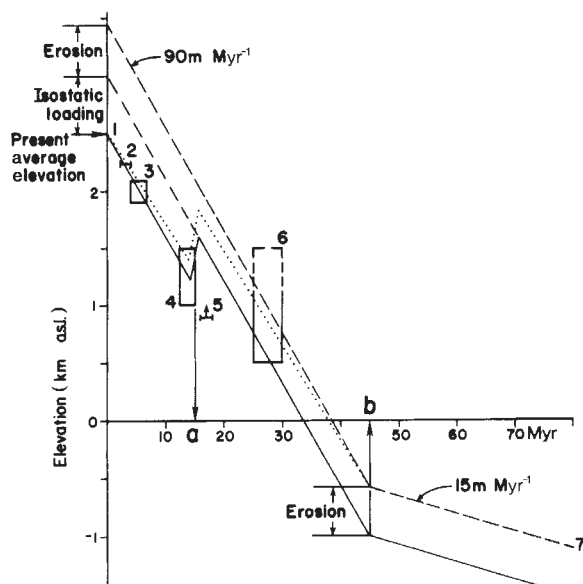


Fig. 3 Crude uplift curve for the Transantarctic Mountains. The curve has been constructed from data sources which are given in Table 1. The curve starts at the present-day average elevation of the mountains, determined as 2.5 km from a detailed analysis of USGS topographic maps (scale 1:0.25 M). The effects of the development of the east Antarctic ice sheet at about 15 Myr, assuming ice build-up over a period of 200 kyr, would have been to load the crust and depress it by an average amount of 0.5 km². This generates a discontinuity in the uplift curve. Before 15 Myr and back to 45 Myr the estimates, particularly from fission track studies¹⁸, indicate a mean uplift rate also of the order of 90 m Myr⁻¹. The fission track results show a pronounced break at 45 Myr preceded by rate of 15 m Myr⁻¹. Superimposed on the uplift curve are the unknown effects of erosion. Lacking any quantitative determinations of erosion rate in the Transantarctic Mountains a global average value of 10 m Myr⁻¹ has been used, but this is probably a minimum for mountainous terrain. This yields almost 500 m of surface lowering since the beginning of rapid uplift. The arrowed points locate, on the time axis, the approximate inception of a full continental ice sheet in Antarctica (a) and the commencement of the period of rapid uplift (b). Data sources: 1, present average elevation of the mountains; 2, from ref. 24; 3, from ref. 25; 4, from ref. 26; 5, from ref. 27; 6, from ref. 29; 7, from ref. 18.

ice-sheet growth can be dated and related to topography, Antarctica at present gives much more detailed information about its uplift curve than do southern Africa or south-east Australia. Unfortunately, the reverse is true of the history of the inferred extensional zone in Antarctica. Nevertheless, the changes in heat flow and temperature required for the model can be estimated.

A steady-state uplift rate of 90 m Myr⁻¹ (Fig. 3) and a postulated enthalpy for the phase change of 46 kJ kg⁻¹ implies a steady-state heat flux of about 3.5 mW m⁻² (ref. 1). This is about 6% of the average heat flux. Adopting reasonable values for the thermal properties of the lithosphere, a heat flow increment of this magnitude would cause thermal expansion at a rate of ~m Myr⁻¹, which is negligible. Because most of it must go into driving the phase change, the increased heat flux will be virtually impossible to detect from surface measurements. For either the 'basalt-eclogite' or 'granulite-quartz eclogite' phase changes, 4 km of uplift implies that ~30 km of eclogite or quartz eclogite have inverted to the low density form. If the lower continental crust is of intermediate composition, then the granulite-quartz eclogite transformation is more likely²². The inferred 3.5-mW m² heat flux represents some 10–17% of the inferred mantle heat flux in continental regions²³. With a mantle conductivity of 3.4 W m⁻¹ °C⁻¹ (ref. 23), this would cause a temperature increase of about 1 °C km⁻¹, giving a temperature increase of about 110 °C at the base of the lithosphere.

There appears to be no early Cenozoic volcanism associated with the initial overriding of east Antarctica onto the postulated

linear heat source. The nature, timing and approximate position of the late Cenozoic volcanism is reasonably well explained by the model.

Uplifted areas associated with basaltic volcanism like those of south-east Australia and east Antarctica might also enable different reference frames for inferring gross flow patterns in the asthenosphere to be distinguished. In the case of the uplift and volcanism of Antarctica, there is no clear distinction between a frame fixed to South America or the hotspots frame.

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Possible gyre-gyre interaction in the Pacific Ocean

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The subtropical anticyclonic gyres of the North and South Pacific Ocean are among the largest circulation systems found in the ocean. The gyres contain the warm waters of the warm water sphere^{1,2}, and the rotation of these water masses is subject to low-frequency variations³. The presence in these gyres of baroclinic Rossby waves with periods of many years has been documented^{4,5} and this seems to be sufficient proof that the gyres undergo vacillations. Using sea-level data we demonstrate here that the subtropical gyres of the North and South Pacific oscillate with a period of about 4 years and that these oscillations are probably induced by the major El Niño events.

Oscillations of the subtropical gyre in the North Pacific Ocean have been established³, but in the Southern Hemisphere there are insufficient hydrographic data to show that the thermal or density structure of the gyre, or its water volume, undergoes systematic low-frequency variations. There is some evidence in sea-level data of an annual oscillation between the two hemispheres, but this seems to be largely a thermal signal restricted to the surface layer⁶. Sea level along the coasts of South Australia and California shows low-frequency correspondence, which has been linked with heat and water exchange between the Pacific and the Indian Oceans through the Indonesian Waters⁷.

During 1974–75 a network of sea-level gauges was established on islands in the Pacific to monitor sea-level variations⁸. Monthly mean values of sea level during the period 1975–81 were analysed by using empirical orthogonal functions⁹. The first of these functions, accounting for 42% of the non-seasonal variance, reflects an east–west oscillation of the equatorial Pacific, and its time-dependence is related to the 1976 El Niño event. Its spatial distribution closely resembles the model calculations of McCreary¹⁰. The second function, accounting for 11% of the variance, constitutes a north–south oscillation across the Equator. It has maximum opposite amplitudes around the Hawaiian Islands in the North Pacific and in the vicinity of Samoa and the Solomon Islands in the South Pacific. The oscillation goes through slightly more than one full cycle during the 7-yr period covered by the data, and its identification led to analysis of longer time series.

To investigate the oscillation further, the longer sea-level records at Honolulu (21° N 158° W, 1905–82) and Pago Pago (14° S 171° W, 1949–82) were subjected to a spectral analysis for the common period of data (Fig. 1). Spectral energy is high at low frequencies with periods between 3 and 4 yr. Highest coherence (0.74) between Honolulu and Pago Pago in the low-frequency range is found at a period of about 4 yr and is significant at the 87% confidence level. The oscillation at the two stations is almost completely out of phase—Pago Pago leads Honolulu by 160°. This analysis indicates that the two gyres fluctuate out of phase with typical periods around 4 yr, which is also evident from the original records and their difference (Fig. 2). Typical amplitudes of these fluctuations are 5–8 cm at each station.

We interpret the observed sea-level fluctuations as oscillations among the two large subtropical gyres of the Pacific. Each gyre

constitutes a mass of warm water, which is deepest in the subtropics and near the western side of the ocean. This water mass rotates in an anticyclonic direction. If the rate of rotation or angular momentum of the gyre changes, the associated density structure must adjust because of the geostrophic balance. The

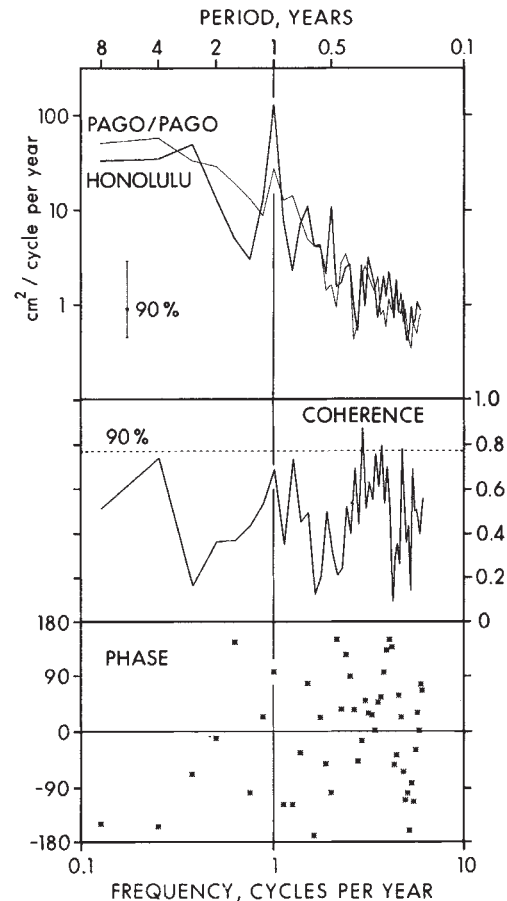


Fig. 1 Spectra of monthly sea level at Honolulu and Pago Pago for the period 1951–82, their coherence and phase for frequencies between 10 and 0.1 cycles yr⁻¹.

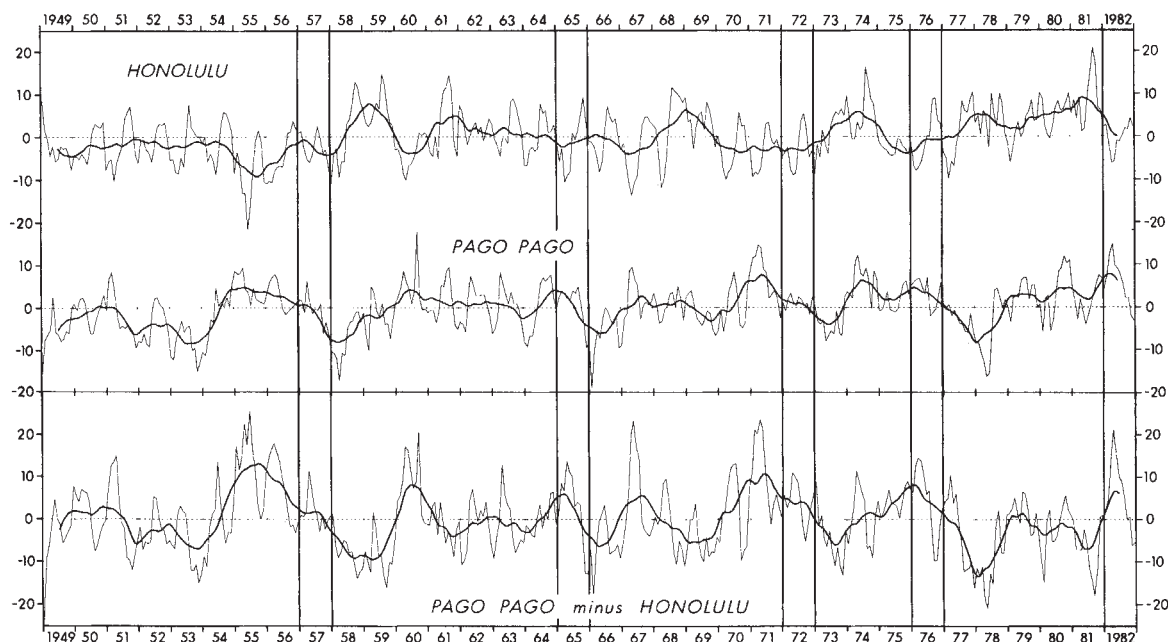


Fig. 2 Monthly mean sea level at Honolulu and Pago Pago and their difference (in cm) from 1949 to 1982. The thick curves give the 12-month running means. The major El Niño events of 1957, 1965, 1972, 1976 and 1982 are indicated by vertical lines.

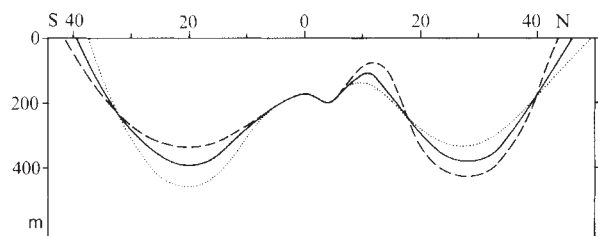


Fig. 3 The shape of the thermocline across the two subtropical gyres (solid line) and possible modes of fluctuations (dashed and dotted lines).

nature of this adjustment is sketched in Fig. 3; an acceleration of the circulation would depress the thermocline in the centre and raise it along the periphery of the gyre. A deeper thermocline implies a higher sea level because of the hydrostatic relation. If the relative density contrast between the upper layer of the gyre and the deep ocean $\Delta\rho/\rho$ is 3×10^{-3} , the depth of isopycnals in the thermocline would change 20 m for a sea-level change of 6 cm. With a depth of the gyre of 500 m, the change in the geostrophic transport of the gyre corresponding to a sea-level change of 6 cm would be $\sim 4 \times 10^6 \text{ m}^3 \text{ s}^{-1}$ or about 10% of the gyre transport. Hydrographic data are inadequate to detect such small low-frequency changes in the depth of isopycnals, but transport fluctuations of similar magnitude in the North Pacific gyre have been documented³.

Three basically different possibilities exist for the adjustment.

- (1) If the mass of water in each gyre remains constant, the two gyres might vacillate independently and with different periods.
- (2) If water is exchanged between the two gyres, this exchange has to occur across the Equator and the two gyres would be necessarily coupled. In this case the gyres have to oscillate out of phase, as is observed. (3) It is also possible that the two gyres are dynamically coupled and oscillate without exchanging mass.

It is difficult to speculate about the forcing of the oscillation. It is possible that wind torque over one of the gyres accelerates its circulation. If the two gyres were dynamically coupled, this could lead to the acceleration of the other gyre, but the oscillation would be appreciable only if a natural resonance frequency existed. This possibility could be investigated by a hydrodynamical model and the computation of the free oscillations of such a two-gyre system. On the other hand, a trans-equatorial water transport could force a coupling between the two gyres, but this coupling would not necessarily cause an acceleration or deceleration of the gyres.

The oscillations of sea level at Pago Pago (Fig. 2) follow a definite pattern related to major El Niño events. On each occasion sea level is high before El Niño, sea level drops during El Niño and is low near the end of and immediately after El Niño. In contrast, sea level at Honolulu does not show such a clear relation to El Niño events, but sea level at Honolulu peaks between such events, namely in 1959, 1968, 1974 and 1981. The difference of sea level between Pago Pago and Honolulu reflects the oscillation even better and shows its relation to major El Niño events. During each El Niño the sea-level difference is high before the event, drops during the event and is low after the event. This is the case for the major El Niño events of 1957, 1965, 1972 and 1976. Similar weaker fluctuations occur around 1960 and 1967, at a time when no El Niño occurred, indicating that the 4-yr oscillation among the two gyres is a phenomenon separate from El Niño, but might be triggered by it.

It seems possible that the north-south oscillation between the two subtropical gyres is triggered by the massive redistribution of warm water during major El Niño events. Sea-level observations of the 1982–83 El Niño show that warm water drained from the western Pacific north of the Equator during the second half of 1982 and caused a first peak of sea level in December 1982 at the Galapagos Islands. During the first half of 1983 warm water drained from the area between the Solomon Islands and Tahiti, causing a second peak in sea level at the Galapagos Islands in May 1983. During the early part of 1983 the sub-

tropical anticyclonic gyre of the South Pacific was apparently displaced south by 10° latitude¹¹. It is likely that major El Niño events trigger the oscillation among the two subtropical gyres, which continues in the years after the triggering until a subsequent El Niño event starts the cycle again. A more definite answer to this problem must await a dynamical model simulating the interaction of the two gyres.

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¹⁰Be-dating of a manganese crust from Central North Pacific and implications for ocean palaeocirculation

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One of the most promising applications of the new ¹⁰Be detection technique using accelerator mass spectrometry is the determination of growth rates of Mn-nodules and crusts^{1–5}. Because the half life of cosmogenic ¹⁰Be (1.5 Myr) is of the same order as the time needed for a few millimetres of nodule material to grow, ¹⁰Be is a useful tool for unravelling such evolution during the late Tertiary, when most nodules are assumed to have started their growth⁶. Mass spectrometric measurement of a ¹⁰Be profile across the manganese crust VA 13-2 from the Central Pacific, using a tandem van de Graaff accelerator, yields growth rates of 2.7 and 4.8 mm Myr⁻¹ for the layers of the crust accumulated between Recent and 6 Myr BP and between 6 and 11 Myr, respectively. Using these measurements as well as ²³⁰Th dating, we have been able to distinguish boundaries between zones in the crust with different structures and chemical compositions and to assign ages to them. These ages are 0.12, 2.9–3.4, 5.7–6.7, 7–9, 10–12 and 13–16 Myr BP. All of these boundaries apparently coincide with the times reported for Quaternary and late Tertiary palaeoclimatic events, suggesting that the crust growth has been strongly influenced by palaeoclimate.

During RV *Valdivia* cruise VA 13-2, 1976, a very thick ferromanganese crust (20–40 cm) was dredged near the summit of a submarine peak at a depth of 4,830 m in the Central Pacific^{7,8}. In this crust, which directly overlies altered basaltic rock, three macroscopically distinguishable main zones (top zone, middle zone and bottom zone) and numerous subzones T-1 to T-6, M-1 to M-7 and I-1 to I-6 (from top to bottom) have been identified. The individual subzones, ~1–3 cm thick, are built up of macrolayers and microlayers showing a knob-like to cusped texture similar to ferromanganese nodules. It was suggested that in the top zone (T-1 to T-6), where the ¹⁰Be profile was measured, the Mn, Fe, Ni and Co and other trace elements came mainly from seawater. The Mn/Fe ratio indicates hydro-genetic growth all through the top zone⁹, yet the content of Mn, Fe and of trace elements changes from one subzone to another.

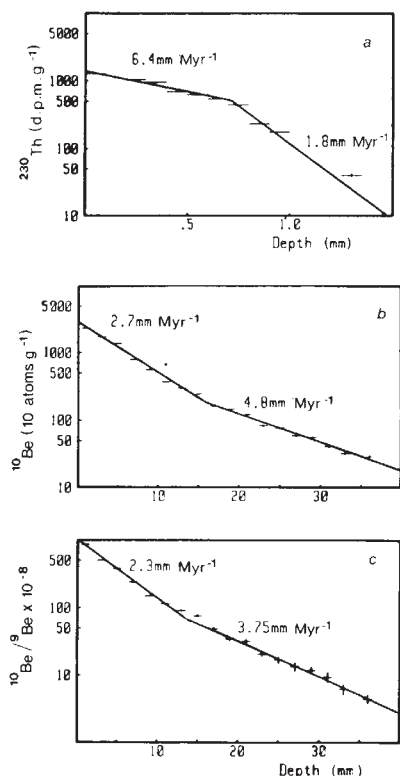


Fig. 1 *a*, ^{230}Th profile for the uppermost 1.4 mm of the crust. The exponential decrease yields growth rates of 6.4 mm Myr^{-1} for the uppermost 0.8 mm of the crust and 1.8 mm Myr^{-1} between 0.8 and 1.4 mm. *b*, ^{10}Be profile for the top zone of the crust. For the section between 0 and 16 mm the growth rate amounts to 2.7 mm Myr^{-1} , and between 16 and 38 mm to 4.8 mm Myr^{-1} . *c*, $^{10}\text{Be}/^9\text{Be}$ ratio for the top zone. It yields growth rates of 2.3 mm Myr^{-1} for the uppermost 16 mm and 3.75 mm Myr^{-1} for the depth-range 16–38 mm.

A closer look at the photomicrograph of polished sections (Fig. 3) reveals the existence of inner structure in excess of the one described by the T-1 to T-6 notation. This structure consists of a sequence of zones with 'pillar' or 'columnar' growth⁸, labelled C1 to C3 and P1 to P3. We observe, for example, a clear change from pillar structure P1 to the columnar structure C2 at a depth of ~16 mm. A detritus-filled layer 16 mm from the top of the crust can be seen on the photomicro. Further layers are located at 6.5 and 22 mm (ref.8).

Samples for the ^{230}Th and ^{10}Be profiles as well as for chemical analyses of Mn, Fe, Co, ^9Be and Ni were taken perpendicularly to the surface using a drill bit of 1.0 cm^2 area, giving a continuous profile through the crust. Be and Th were purified using wet chromatography techniques^{2,3,10}. Th was electroplated and estimated by α -spectrometry. Be was ignited to form BeO and the $^{10}\text{Be}/^9\text{Be}$ ratios of 0.5 to 2 mg BeO samples were measured by mass spectrometry using the EN-tandem accelerator of the ETH-Zürich^{11,12}. The stability of the mass spectrometer was verified by measurement of an internal standard²¹; reproducibility is better than 3%, but the absolute calibration has an uncertainty of ~10%. The sensitivity depends on the ^{10}Be contamination, which for most samples was at the p.p.m. level, leading to a background corresponding to a $^{10}\text{Be}/^9\text{Be}$ ratio of 10^{-14} . Growth rates were derived by applying half lives of 75,000 yr (^{230}Th) and 1.5 Myr (^{10}Be) and under the basic assumptions that: (1) ^{230}Th profiles and $^{10}\text{Be}/\text{mass}$ ratios or the $^{10}\text{Be}/^9\text{Be}$ ratio have been constant throughout time; (2) diffusion is negligible. In other words we assume that the profiles reflect only radioactive decay and crust growth.

^{230}Th yields a growth rate of $6.4 \pm 0.5 \text{ mm Myr}^{-1}$ for the uppermost 0.8 mm of the crust. Between 0.8 and 1.4 mm the growth rate amounts to $1.8 \pm 0.12 \text{ mm Myr}^{-1}$ (Fig. 1*a*). The significant change in growth rate at 0.8 mm is dated at 125,000 yr BP. The

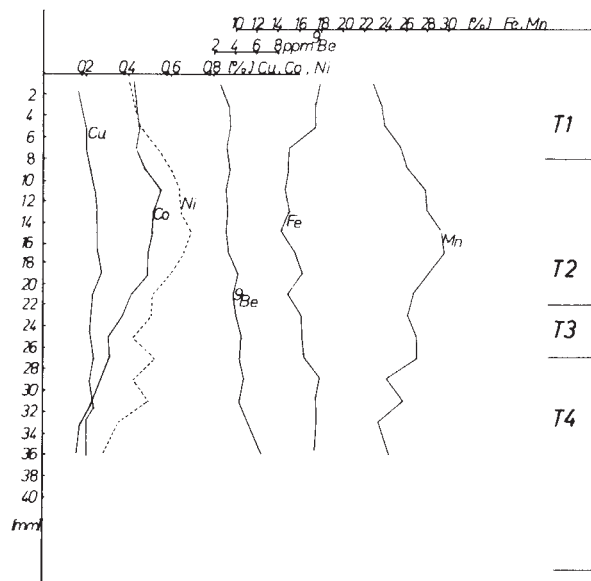


Fig. 2 Chemical composition of the top zone. The Mn content as well as the Ni content has a maximum at a depth of ~16 mm. ^9Be shows a very slight but constant decrease from 4 p.p.m. at 30 mm to 3 p.p.m. at the surface, explaining why growth rates derived from the $^{10}\text{Be}/^9\text{Be}$ depth-profile are 20–30% smaller than those derived from the specific ^{10}Be content.

Table 1 Ages for boundaries of crust sections with different mineralogical structure

Boundary no.	Depth (mm)	Age (Myr)
1	0.8	0.125 ± 0.01
2	1.4	0.46 ± 0.04
C1/P1	8.0	2.9–3.4
P1/C2	15.5	5.7–6.7
C2/P2	23.0	7.8–9.1
C3/P3	36.0	10.0–12.0
Bottom of T6	42.0	11.0–14.0

Growth rates were derived from the ^{230}Th profile for the upper 1.4 mm and from the ^{10}Be -profile and the $^{10}\text{Be}/^9\text{Be}$ -ratio respectively for the deeper layers. The uncertainties of the ages result from the different growth rates calculated from the $^{10}\text{Be}/\text{mass}$ and $^{10}\text{Be}/^9\text{Be}$ -ratios, respectively (see Fig. 2).

^{10}Be , as well as the $^{10}\text{Be}/^9\text{Be}$, depth profiles (Fig. 1*b, c*) show an exponential decrease and a clear change of the growth rate at 16 mm. The growth rate for the section 0–16 mm amounts to $2.7 \pm 0.13 \text{ mm Myr}^{-1}$ and $2.3 \pm 0.2 \text{ Myr}^{-1}$, as derived from the ^{10}Be and $^{10}\text{Be}/^9\text{Be}$ profiles. For the section between 16 and 38 mm we derive growth rates of 4.8 ± 0.2 and $3.75 \pm 0.25 \text{ mm Myr}^{-1}$, respectively. Applying these rates we are able to calculate ages for the boundaries of the crust sections with different texture as shown on Table 1 and Fig. 3. For comparison with other crust data from the Central Pacific^{1,5,13}, we compute fluxes of ^{10}Be and ^{230}Th on the surface of the crust to be 1.4×10^7 atoms ^{10}Be per cm^2 per 10^3 yr and $1.2 \text{ d.p.m. per cm}^2$ per 10^3 yr, derived as $F = G \times C \times D$, where G is the growth rate, C the element concentration at the surface and D the bulk density¹³.

The smooth exponential decrease of the $^{10}\text{Be}/\text{mass}$ and $^{10}\text{Be}/^9\text{Be}$ profiles for individual larger sections as found on the crust VA 13-2 implies constant growth rate and ^{10}Be deposition. The results suggest constant $^{10}\text{Be}/\text{carrier mass ratio}$ for the past 11 Myr, the standard deviation from an exponential is <5%. This is a similar conclusion to Ku *et al.*⁵ who reported evidence for constant deposition of ^{10}Be and ^9Be during the past 7–9 Myr for two analysed crusts from the Pacific and Atlantic Oceans. Further support for a constant ^{10}Be to mass ratio derived from VA 13-2 is (1) the numbers fit well Turekian *et al.*'s plot of the ^{10}Be flux against nodule growth rate on ^{10}Be dated nodules,

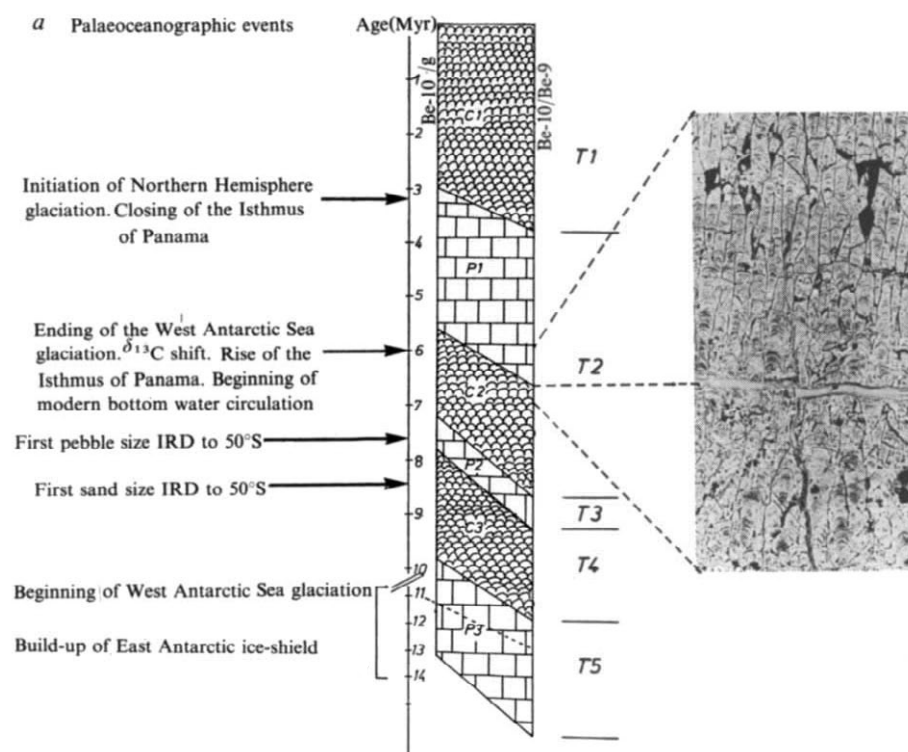


Fig. 3 *a*, Ages for the different sublayers of the top zone, calculated from the ^{10}Be -profile and from the $^{10}\text{Be}/^9\text{Be}$ ratio, respectively, and the palaeoceanographic time-markers during late Tertiary and early Quaternary. The uncertainties of the ages reflect the different growth rates derived from $^{10}\text{Be}/\text{mass}$ and $^{10}\text{Be}/^9\text{Be}$ ratios. *b*, Section of the photomicrograph showing the change from pillar to columnar structure P1 to C2 at 16 mm depth.

suggesting proportionality; (2) a 1.8-fold increase of the growth rate below 16 mm leads only to a different slope of the ^{10}Be depth profile and not to a significant offset of the specific ^{10}Be concentration.

One explanation would be to assume that in the water column the Mn carrier adsorbs an equilibrium amount of ^{10}Be , which is only a comparatively small fraction of the total available ^{10}Be ($\approx 2.5\%$). Normalization of ^{10}Be against ^9Be has the advantage of making dispensable the assumption of a constant ^{10}Be to carrier ratio. It assumes, however, a constant ^9Be input to the open ocean and a constant concentration in seawater throughout time which, due to the comparatively short residence time of Be in the ocean, might be even more questionable than the constant $^{10}\text{Be}/\text{mass}$ assumption. A further crucial question is the extent to which the time record derived from the ^{10}Be depth profile may be distorted by diffusion within the crust. As has

been pointed out earlier, diffusion, or diffusion associated with growth could also yield exponential depth profiles^{3,4}. If we assume for the efficient diffusion coefficient of ^{10}Be an upper limit of $6.4 \times 10^{-9} \text{ cm}^2 \text{ yr}^{-1}$ (ref. 14 and A.M., M.S., H. Kudräß, G.B., H.J.H., M.N., M.S. and W.W. in preparation), then $<20\%$ of the slope in crust VA 13-2 should be ascribed to diffusion, requiring a proportional correction of the time scale derived.

The data imply that the inner structure of the Mn crust may reflect global palaeoceanographic events. We shall therefore now discuss ages derived from VA 13-2 by taking as a frame of reference the comprehensive compilation of major Miocene to mid-Pleistocene events and episodes of Ciesielski *et al.*¹⁵.

Sublayer T-1/T-2: The change from columnar zone C1 to pillar zone P1 is dated to 2.9–3.4 Myr, which corresponds with much evidence for beginning of the Northern Hemisphere glaciation¹⁶.

Sublayer T-2: Structural change from pillar P1 to columnar C2 zone correlates with a different Mn-supply. The age of 5.7–6.7 Myr relates it to a most remarkable shift in $\delta^{13}\text{C}$ in the CaCO_3 in the Pacific Ocean dated to 6.2 Myr (refs 17–19). At this time the West Antarctic Sea glaciation ended giving rise to what is presumed to be the beginning of the modern bottom water circulation. Tectonic activities led to a rise of the Isthmus of Panama. This date also lies within the time period 7.4–6.2 Myr where Ciesielski *et al.*¹⁵ assume that the main phase of erosion on the Maurice Ewing Bank occurred. Most remarkable at this boundary is the change of the growth rate as determined from ^{10}Be : this change in the growth pattern seems not to be an isolated phenomenon for VA 13-2. On the SCHW-1D crust from the Pacific dated by Ku *et al.*⁵, the age of 6 Myr corresponds to layer 9.6 mm from the surface and lies within their sample 12 (9.12–10.7 mm). The flatter slope for a fit to the samples below 12 suggests that a shift in growth rate has occurred.

Evidence for a change in growth pattern at around 6 Myr BP is also recorded in several other Mn-encrustations from the North, Central and South Pacific¹⁴.

Beyond sublayer T-2 there are three further clearly visible boundaries and changes from pillar and columnar structures that we date to 7.2–8.6, 7.8–9.1, and 10–12 Myr. Extrapolation of the ^{10}Be depth profile to 43 mm depth gives an age of 13–16 Myr BP for the basis of the top-layer. The first two markers in time could coincide with the occurrence of first pebble and

Table 2 Analytical data for Mn, Fe, Co, Ni, ^9Be and ^{10}Be and the $^{10}\text{Be}/^9\text{Be}$ ratio in the top zone of the crust VA 13-2

Depth (mm)	Mn (%)	Fe (%)	Co (%)	Ni (%)	Cu (%)	^9Be (p.p.m.)	^{10}Be ($\times 10^7/\text{g}$)	$^{10}\text{Be}/^9\text{Be}$ ($\times 10^{10}$)
00–02	23.02	17.95	0.43	0.41	0.16	2.7	$2,320 \pm 70$	142.0
02–04	23.80	17.49	0.44	0.43	0.18	3.5	$1,751 \pm 30$	83.0
04–06	24.16	17.49	0.45	0.46	0.20	3.6	$1,384 \pm 40$	63.0
06–08	25.62	15.02	0.44	0.54	0.20	3.3	791 ± 25	39.8
08–10	26.21	15.08	0.48	0.60	0.22	3.6	560 ± 15	25.8
10–12	27.94	14.73	0.55	0.64	0.24	3.2	370 ± 10	19.2
12–14	28.19	15.12	0.52	0.65	0.25	3.3	300 ± 13	15.1
14–16	29.39	14.28	0.51	0.69	0.25	3.2	244 ± 10	12.7
16–18	29.64	15.47	0.49	0.66	0.25	3.3	162 ± 11	8.2
18–20	28.22	16.20	0.49	0.60	0.26	4.2	146 ± 10	5.8
20–22	26.70	14.91	0.41	0.51	0.23	3.8	122 ± 15	5.3
22–24	26.20	16.10	0.37	0.50	0.22	4.0	83 ± 5	3.4
24–26	26.98	16.19	0.30	0.42	0.22	4.5	77 ± 8	2.8
26–28	27.03	16.42	0.31	0.52	0.23	4.3	59 ± 9	2.3
28–30	24.15	17.85	0.26	0.42	0.21	4.7	56 ± 4	2.0
30–32	25.76	17.49	0.22	0.49	0.22	4.3	41 ± 5	1.6
32–34	23.40	17.55	0.17	0.35	0.20	5.1	32 ± 3	1.0
34–38	24.40	17.26	0.15	0.28	0.19	6.4	29 ± 2	0.7

Recent cross-calibrations showed that the Zürich Internal Standard yields 38% lower ^{10}Be concentrations than the standard at Rutgers University.

sand-size ice-rafted detritus to 50° S, on the Maurice Ewing Bank¹⁵, which are considered to be an indicator for increased Antarctic Circumpolar Circulation (ACC). The growth of the top-zone apparently began with the build-up of the East-Antarctic ice-shield²⁰.

Finally, note that at sublayer T-1, at 0.8 mm from the surface ($\approx 125,000$ yr BP), where ²³⁰Th indicates a remarkable variation in growth pattern, Mn begins a steady increase from $\approx 27\%$ (the average for the uppermost 42 mm) to $\approx 35\%$ by weight at the surface⁸. This might indicate a different and larger supply of Mn in the most recent history of the crust. Although the micro-probe analysis shows that this is one of the largest variations of the Mn concentration over the last 11 Myr, we have to be aware that there might have been other short-term rate variations which we are not able to determine at the present stage of sampling resolution for ¹⁰Be ($\approx 800,000$ yr).

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A relationship between dunes, fire and climate recorded in the Holocene deposits of Quebec

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During the past 5,000 yr BP the Tropics have experienced an increase in aridity and aeolian activity in both maritime and continental areas^{1–4}. In contrast the onset and subsequent development of post-Hypsithermal climates in northern latitudes were primarily conducive to global cooling. It is suggested here that aeolian activity in cold environments represents a direct response to this long-term cooling trend that began after 6,000–5,000 yr BP, at the end of the climatic optimum^{5–9}. This study establishes the Holocene chronology of sand dunes near the outer forest limit in northern Quebec (Fig. 1). I show here that fires initiate the aeolian activity, and that the fire–dune relationship is typical of cooling period. On this basis, a palaeoclimatic reconstruction since the climatic optimum is proposed.

The chronology of aeolian activity was based on 101 ¹⁴C dates. Eighty-six organic horizons were sampled from dune palaeosols that contained discrete, non-mixed, coniferous and shrub-species charcoal, and the remaining samples were non-charred wood fragments found in dune blow-outs. The ¹⁴C histogram (¹⁴C yr) was constructed using the methods of Ochietti and Hillaire-Marcel¹⁰. Aeolian phases were established considering both the cumulative statistical weight frequency of ¹⁴C dates and the distribution of individual ¹⁴C dates (Fig. 2).

The histogram (Fig. 2) indicates that the aeolian activity in the study area over the past 5,000 yr BP occurred episodically. Aeolian recurrences are confined to 13 periods: 4,650, 4,200, 3,850, 3,550, 3,125, 2,875, 2,425, 2,075, 1,775, 1,475, 1,175, 575 and 175 yr BP. The time interval between aeolian activity peaks varied from 250 to 450 ¹⁴C yr, except between 1,175 and 575 yr BP where it was about 600 ¹⁴C yr. The histogram shows three major aeolian intervals: 3,250–2,750 yr BP, 1,650–1,050 yr BP, and 750 yr BP to present (these periods are shaded on Fig. 2). The last period was interrupted for a short time around 350 yr BP. Because of the limited number of ¹⁴C dates, similar long-lasting periods cannot be confirmed before 3,300 yr BP.

Most of the dune palaeosols were Dystric Brunisols¹¹. If present, the eluviated horizon was thin (Ac or Ae_j), less than 2 cm thick, above a more or less sesquioxide-rich Bm horizon (Fe + Al pyrophosphate). Only one palaeosol was found to present a Bf horizon typical of podsol. Selected soil profiles from the various phytogeographical zones of the study area are shown in Fig. 3. Although variable, illuviated sesquioxides found in dune palaeosols rarely exceeded a value of 0.15%. Higher values were obtained in some palaeosols developed during the 2,200–1,650 yr BP period (Fagnant Lake and Great Whale River sites, Fig. 3), suggesting favourable pedogenic conditions. In all the study sites, the original soil surface was pedogenically developed. In Burton Lake, Little Whale River and Clearwater Lake sites (Fig. 3), it was the bottom soil that presented the highest pyrophosphate values compared with the dune soils found above. The development of a soil profile within the original non-ablated surface is an indication that the deposit remained for some time in an undisturbed state allowing plant colonization and soil development to occur. The time-lag between the original deposit setting and the onset of aeolian activity varied from one site to another, depending on the rates of deglaciation and of the recession of the Tyrrell Sea¹². Accumulation of aeolian deposits above the original surface was the result of an ecological disequilibrium in a previously stable morphosedimentary unit. Some palaeosols were frost-disturbed before their burial. Frost cracks varied from 10 to 125 cm in length within the organic and mineral horizons (such as Richmond Gulf, Fig. 3). These wedge-like cracks ranged between 5 and 10 cm in width at the top, and their vertical configuration was emphasized by iron stainings. They were probably caused by seasonal frost cracks¹³ that were likely to have formed in a single season, in severe cold conditions in snow-free sites. Thawing caused local subsidence and subsequent discordant bedding. Another consequence of geliturbation was observed in some soil profiles as involuted features (Minto Lake, Fig. 3). All these frost structures were observed in the following dated palaeosols: 3,210 ± 90, 3,040 ± 215, 1,780 ± 90, 1,615 ± 100, 1,400 ± 160, 1,275 ± 90, 1,210 ± 80, 1,160 ± 80, and 1,040 ± 160 yr BP. The periods around 3,125 yr BP and between 1,650 and 1,050 yr BP were particularly conducive to frost action in soils. The northernmost sites of the study area (Clearwater Lake, Richmond Gulf, Minto Lake, Fig. 1) presented more frost-disturbed palaeosols than their southern counterparts.

The presence of many buried organic horizons in dune palaeosols suggests an alternation of stabilization stages by the plant cover and erosional stages. The number of alternations progressively decreases from the Boreal Forest Zone to the Arctic Tundra Zone. Higher frequencies (14 in Burton Lake site, Fig. 3) in forested areas (respectively the Boreal Zone and the Forest sub-Zone of the Forest Tundra, *sensu* Payette^{14,15}) are an indication that aeolian activity was episodic and that dune stabilization was easier than in the Tundra Zone. North of the forest limit, dune landforms are the result of a more intense and long-lasting aeolian process¹⁶. In all the study sites, the buried organic horizons contained charcoal (mostly coniferous and shrub remains), suggesting a close relationship between fires and aeolian activity; fire incidence is especially conducive to erosion in rather sandy areas. Although no inference can be made about the absolute fire frequency during the Holocene, the decreasing number of buried horizons from the Boreal Forest

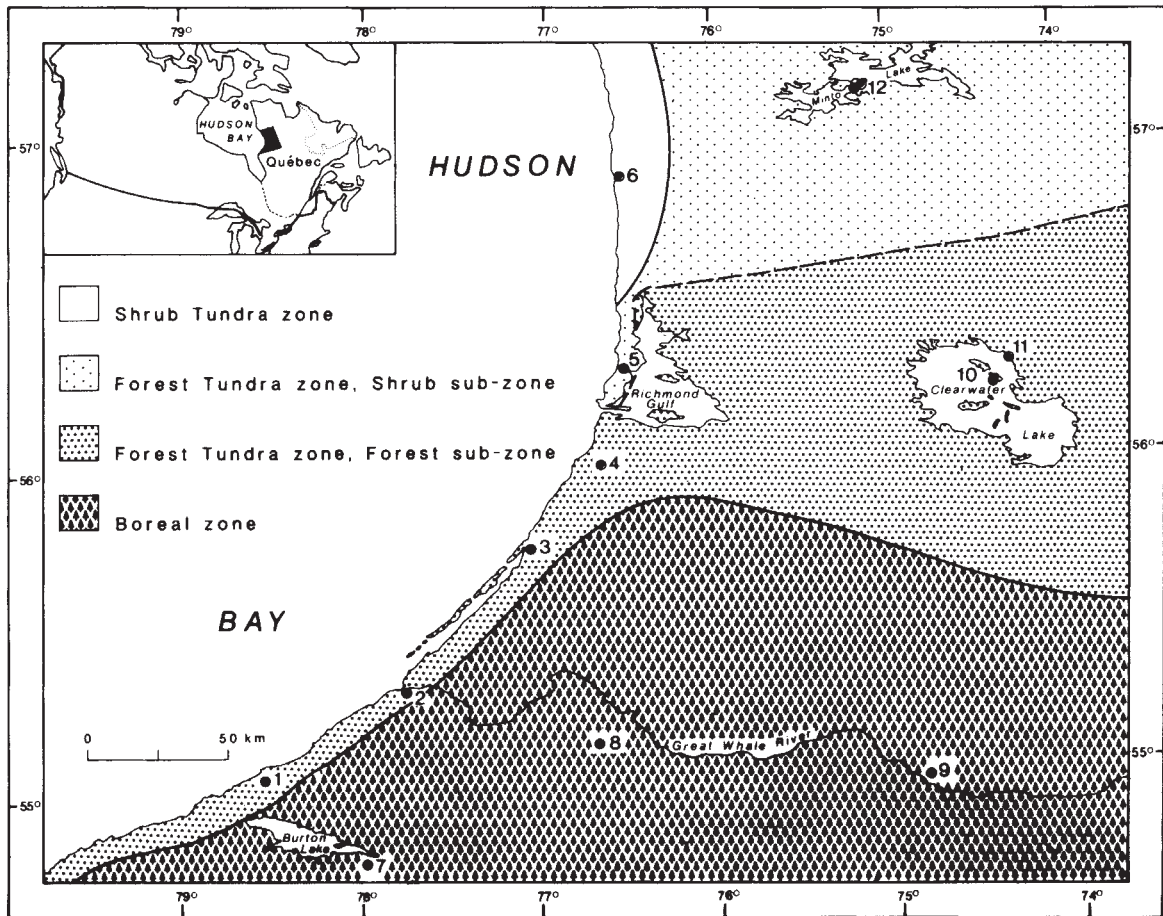


Fig. 1 Location of the study area and phytogeographical zones along the eastern coast of Hudson Bay (study sites: 1, Sucker Creek; 2, Poste-de-la-Baleine; 3, Manitounouk; 4, Little Whale River; 5, Richmond Gulf; 6, Nastapoka; 7, Burton Lake; 8, Fagnant Lake; 9, Great Whale River; 10, Ile-des-Foreurs-Clearwater Lake; 11, Nooniche-Clearwater Lake; 12, Minto Lake).

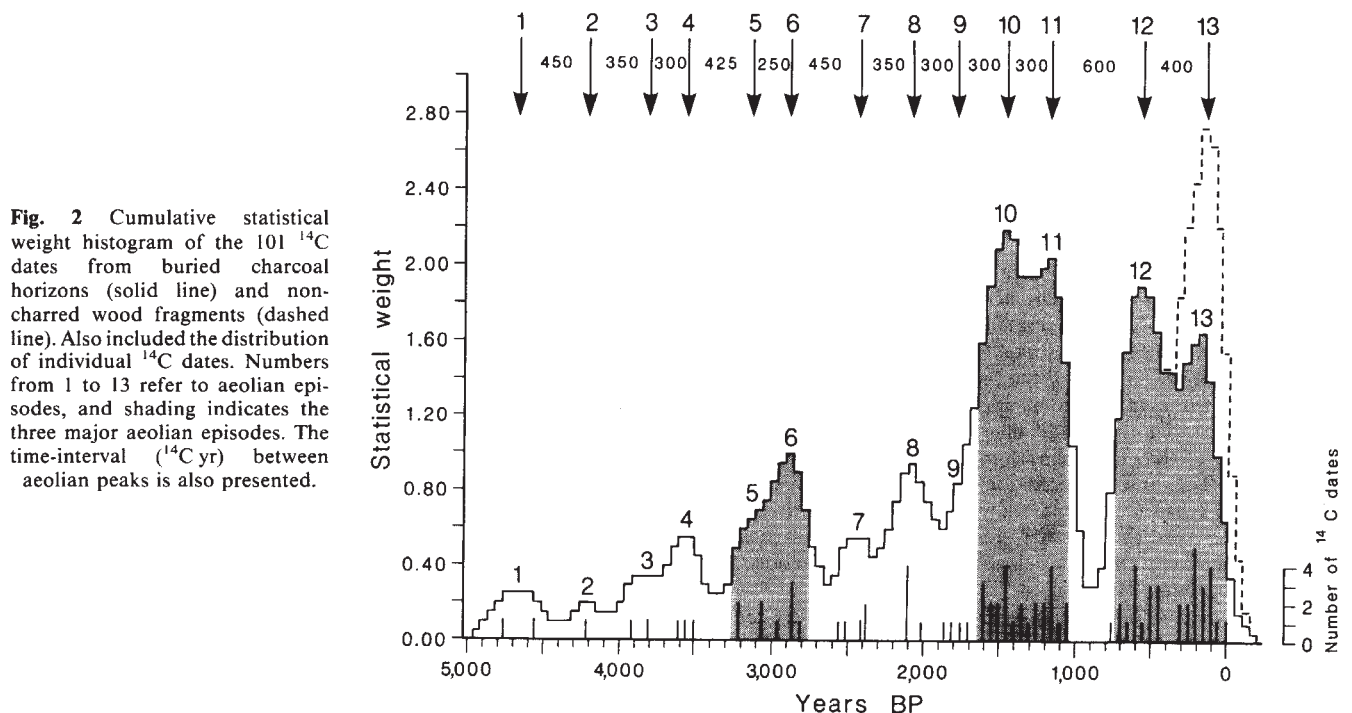


Fig. 2 Cumulative statistical weight histogram of the 101 ^{14}C dates from buried charcoal horizons (solid line) and non-charred wood fragments (dashed line). Also included the distribution of individual ^{14}C dates. Numbers from 1 to 13 refer to aeolian episodes, and shading indicates the three major aeolian episodes. The time-interval (^{14}C yr) between aeolian peaks is also presented.

to the Tundra emphasizes a decreasing gradient in fire incidence. Some long intervals of erosion-free activity, as, for example, in Little Whale River (3,610–1,160 yr BP) and Minto Lake (2,570–1,210 yr BP) sites (Fig. 3), could be the result of a lower fire

frequency north of the forest limit which experienced only minor movements during the Holocene¹⁷. On the other hand, a detailed analysis of the regional chronologies¹⁸ showed that greatest aeolian activity shifted from south to north between ~5,000 and

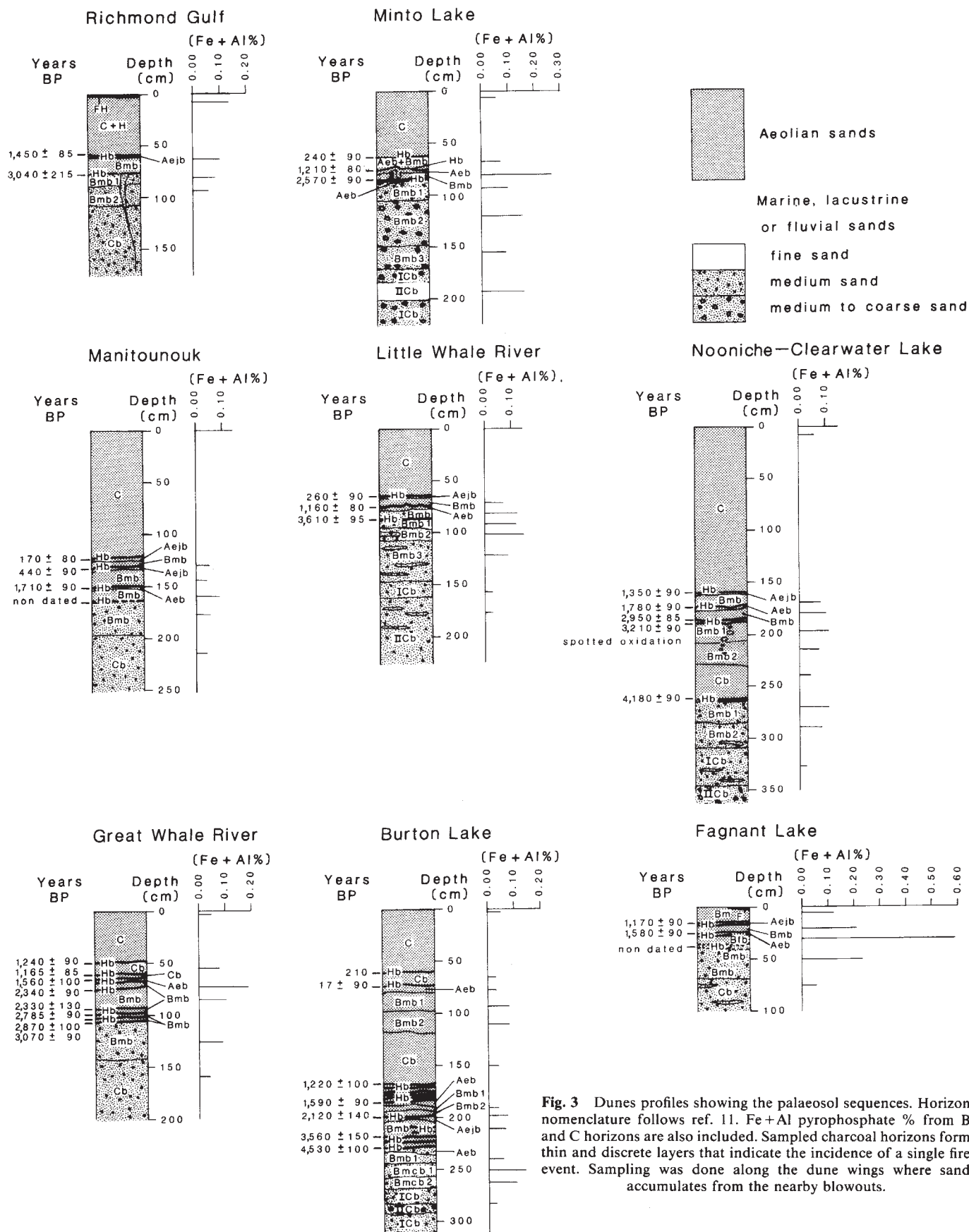


Fig. 3 Dunes profiles showing the palaeosol sequences. Horizon nomenclature follows ref. 11. Fe+Al pyrophosphate % from B and C horizons are also included. Sampled charcoal horizons form thin and discrete layers that indicate the incidence of a single fire event. Sampling was done along the dune wings where sand accumulates from the nearby blowouts.

3,500 yr BP. Aeolian activity first started around 4,650 yr BP in the Boreal Zone sites, around 4,200 yr BP in the Forest Tundra, and finally reached the Tundra Zone around 3,850 yr BP. It thus came soon after the Hypsithermal in the Boreal Zone, whereas

in the Tundra Zone it began only a thousand years later. This diachronism and south-to-north displacement probably resulted from a decrease in fire incidence from the Taiga to the Tundra. The areas located in the Northern Boreal Forest and in the

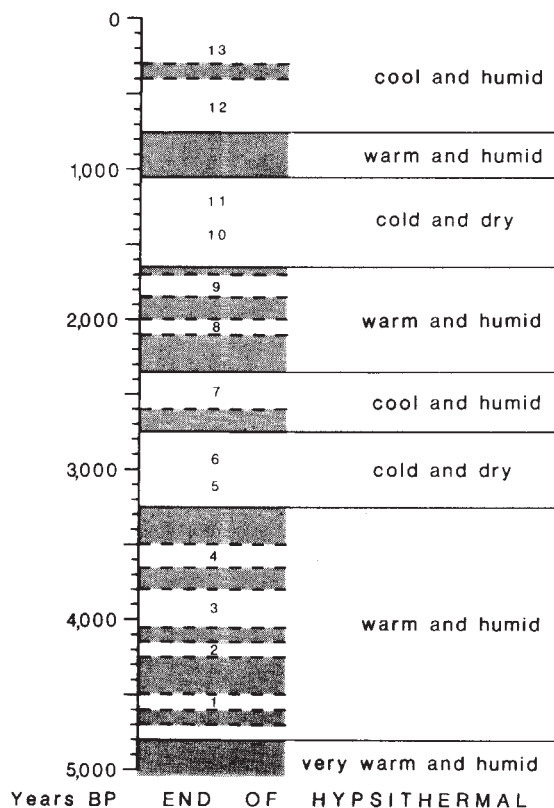
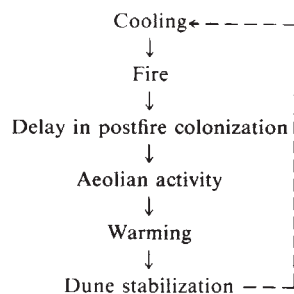


Fig. 4 Palaeoclimate reconstruction since the end of Hypsithermal. The periods delineated by a solid line are characterized by a distinct climatic regime, and the ones with a dashed line correspond to more clement (grey) and to cooling (white) intervals. Numbers 1 to 13 are the aeolian episodes.

Forest Tundra appear most appropriate for the study of aeolian activity during the Holocene, because here the forest biomass can be most conducive to the development of major fires. Here the fire frequency is relatively high, and post-fire colonization (dune stabilization) is more likely to occur during the warming periods and, inversely, may be compromised during cooling periods. Because they modify the structure of the plant landscape, fires influence the environmental equilibrium in such a way as to prevent forest regeneration. They seem to act as catalysts in inducing ecosystem change (for example, forest to dune shift) during cooling periods. This interpretation has been proposed elsewhere with regard to snow-patch expansion in northern Quebec¹⁹, tundra-type vegetation expansion within the Forest Tundra²⁰, and Holocene displacements of the forest limit^{17,21}. The development of aeolian systems in cold environments thus represents another ecological response to the fire-triggering process.

The periglacial aeolian cycle can be summarized as follows:



However, this cyclical model is not applicable to the tundra area. At those latitudes, aeolian systems follow a rather directional (secular) development. Climatic cooling after 3,000 yr BP in the Tundra was so important that dune stabilization was almost impossible even during brief warm periods^{16,18}.

Cold-region aeolian activity responds to the alternation of unfavourable climatic periods (peaks in Fig. 2) and favourable ones (lows in Fig. 2). Cold episodes follow the general, secular climatic cooling trend after the Hypsithermal (~5,000 yr BP), and became more important after 3,250 yr BP. Taking into account the fluctuations of the aeolian activity *per se*, it is possible to establish more precisely the climatic conditions that prevailed during the Holocene. Cold and dry conditions would be conducive to a significant increase of aeolian activity. The similar characteristics found in palaeosols related to the 3,250–2,750 yr BP and to the 1,650–1,050 yr BP periods are suggestive of such conditions. The presence of frost cracks during these periods would indicate severe cold conditions accentuated by a relatively dry climate (snow-free sites or with thin snow cover). Moreover, many palaeosols associated with these periods are related to a set of chronologically closely-bracketed organic horizons, emphasizing sustained erosional conditions probably after each forest fire (Burton Lake, Fig. 3, the 1,590–1,220 yr BP interval). Swain²² also proposed that there were dry conditions in Wisconsin between 1,700 and 1,150 yr BP, based on pollen, macrofossil and charcoal evidence. A cool and humid climate would inversely decrease the aeolian activity. This trend is apparent between 2,750 and 2,350 yr BP (period 6–7, Fig. 2). The 750 yr BP to present period (episodes 12 and 13, Fig. 2) also registered a decrease compared with the 1,650–1,050 yr BP period (episodes 10 and 11, Fig. 2). This trend is more typical of maritime sites (Manitounouk, Little Whale River, Richmond Gulf, Fig. 1). More humid conditions may have accentuated the summer maritime ambiance along the Hudson Bay coast, thus reducing any fire and wind erosion probabilities (soil humidity increase). These two periods were also characterized globally by significant continental and alpine glacier expansions in mid- and high-latitude areas^{6,23–25}, and by snow-patch inception in northern Quebec¹⁹. A humid and warm climate would allow only local aeolian activity (4,800–3,250 yr BP, 2,350–1,650 yr BP) or would prevent it completely (Hypsithermal, and 1,050–750 yr BP). There is no evidence that a combined warm and dry climate ever existed at those latitudes. The palaeoclimatic reconstruction proposed here for the past 5,000 yr BP (Fig. 4), based on fluctuations of aeolian activity in northern Quebec, is presented as a first approximation. Nevertheless, it brings up the possibility of a cool/humid and cold/dry alternation in climate during the Holocene in northern, continental latitudes, as suggested by the long-term development of dune systems.

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A Gunflint type of microfossil assemblage from early Proterozoic stromatolitic cherts in China

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A well-preserved early Proterozoic microfossil assemblage dominated by a filamentous form comparable with *Gunflintia minuta* Barghoorn and a coccoid form comparable with *Huroniospora* Barghoorn has been discovered in silicified stromatolitic cherts of the Dahongyu Formation, Changcheng Group, northern Hebei Province, China. It is the oldest *in situ* microfossil assemblage in China. The famous Gunflint fossils, from the Gunflint Iron Formation, Ontario¹, were the first well preserved and relatively diverse Precambrian microbiota to be described^{2,3}. Several other Precambrian microfossil assemblages are now known, but the Gunflint type has thus far been found in only a few other localities: the Duck Creek Dolomite, northwestern Australia⁴; the Frere Formation, western Australia⁵; and the Tyler Formation, northern Michigan, USA⁶. They are all early Proterozoic. The discovery indicates that the stromatolitic benthic microbiota of the Gunflint type had a cosmopolitan distribution in time between 2,000 and 1,700 Myr ago, and may have both palaeoecological and stratigraphical significance.

The sequence forms part of the northern North China Platform (Fig. 1). The fossil locality and the local geology have been described in detail elsewhere⁷. A generalized stratigraphy is given in Fig. 2. The early Proterozoic Changcheng Group consists of five formations (from the base up): (1) the Changzhougou Formation (conglomerate, sandstone and quartzite; ~170 m thick); (2) the Chuanlinggou Formation (oolitic and stromatolitic iron formation interbedded with carbonaceous shales; ~60 m thick); (3) the Tuanshanzhi Formation (dolomite, ~170 m thick); (4) the Dahongyu Formation (cherty dolostone, sandstone and lava; 110 m thick); (5) the Gaoyuzhuang Formation (cherty dolostone, ~1,000 m thick).

The Dahongyu Formation is composed of cherty dolostone, littoral to neritic sandstone, arkosic sandstone, tuffaceous sandstone-siltstone, volcanic breccia and potassium-rich trachyte. Several well preserved and distinct microfossil assemblages have been found in the lower and upper parts of this formation. The Gunflint type of microbiota occurs in the lower part of the Dahongyu Formation, in a zone 170–200 m above the top of the Chuanlinggou Iron Formation (Fig. 2). Glauconite from the middle part of the Dahongyu Formation in Jixian (eastern Hebei Province) has K–Ar ages of 1,621, 1,643 and 1,678 Myr. The U–Pb isochron ages of the upper part of the Tuanshanzhi Formation and the Chuanlinggou Formation (the underlying

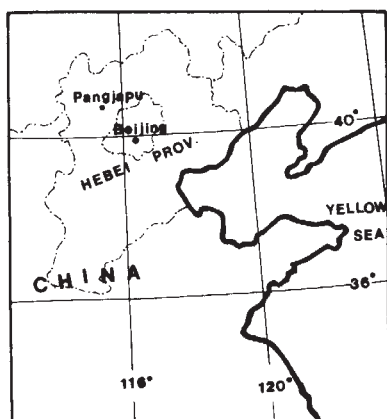


Fig. 1 Location of Pangjapu where the Gunflint type microbiota were discovered.

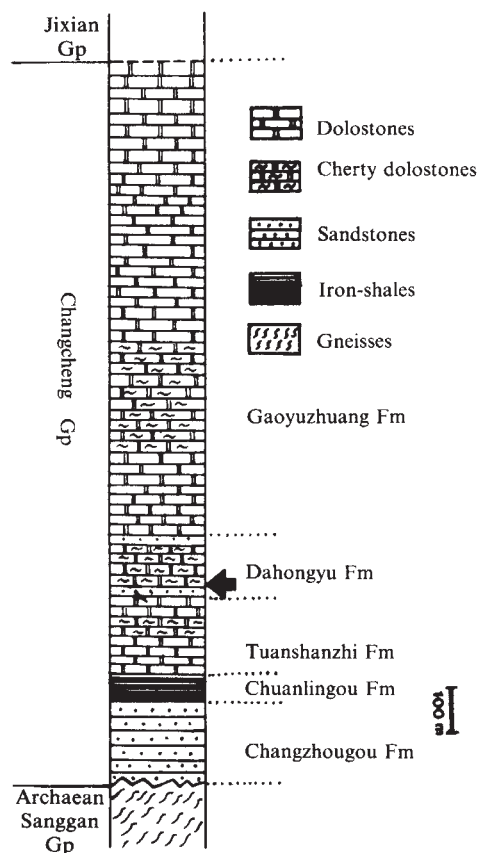


Fig. 2 Stratigraphical column of the early Proterozoic Changcheng Group in Pangjapu region. The arrow shows the position of the Dahongyu microbiota.

sediments) are 1,770 and 1,910 Myr respectively⁸. The age of the Dahongyu microbiota is therefore between 1,650 and 1,770 Myr.

The filaments of *G. minuta* and coccoids of *Huroniospora* sp. are usually found in stromatolitic organic-rich laminae. The filaments are gathered in bunches or intertwined with each other, often forming a network-like fabric (Fig. 3a, b, f, k). The coccoids are mostly scattered in spaces between the filaments. The distribution pattern of the Dahongyu microfossils is thus the same as that of the Gunflint stromatolitic microbiota from Schreiber, Ontario, suggesting that *G. minuta* and *Huroniospora* spp. were the principal mat builders.

The filaments of *G. minuta* from the Dahongyu Formation have diameters of 1–2 μ m and are morphologically very similar to those from the Gunflint Iron Formation of Canada. The so-called 'heterocystous structures', that is occasional bulges in the filaments^{1,9}, have also been observed (Fig. 3e, f, h). It is possible that these structures were not original features of the *Gunflintia* organism, but instead have been caused by cell degradation. 'Budding' similar to that observed in the Gunflint Iron Formation¹⁰ has been observed in the coccoids of the *Huroniospora* type from the Dahongyu Formation (Fig. 3g).

In addition to the two dominant forms mentioned above, some other rare filamentous and coccoid forms have been found (Fig. 3i, j). Some (Fig. 3j) can be assigned to Gunflint taxa such as *Leptoteichos golubicii* Knoll, Barghoorn et Awramik.

Two different facies of fossiliferous cherts have been recognized in the Gunflint Iron Formation: the stromatolitic chert facies containing the *G. minuta*–*Huroniospora* spp. dominated assemblage and the non-stromatolitic chert facies containing the *Kakabekia* and *Eoastrion* dominated assemblage¹. The Dahongyu microbiota, dominated by *G. minuta* and *Huroniospora* spp., more closely resembles the stromatolitic benthic facies^{1,2}.

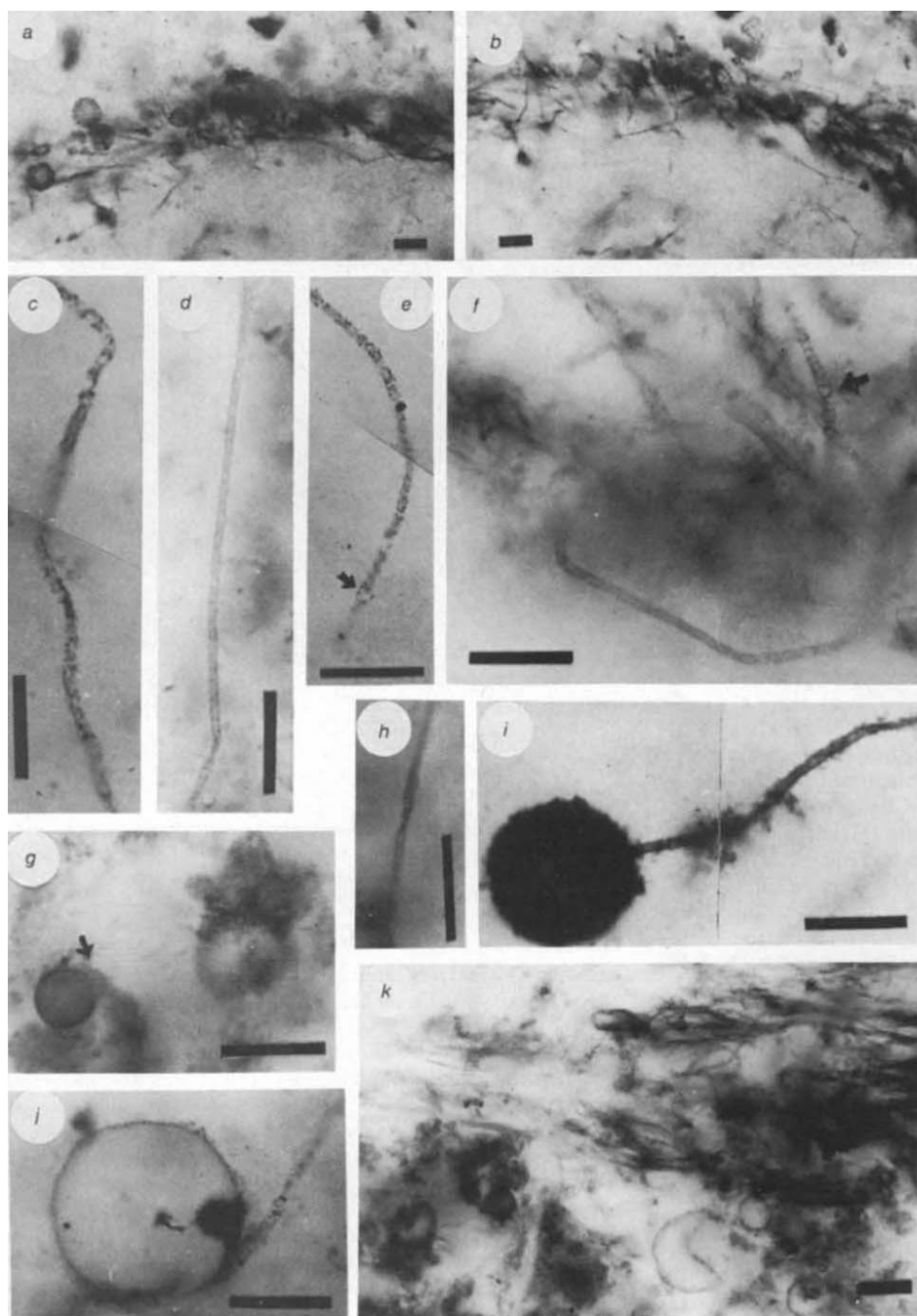


Fig. 3 Microfossils from the Dahongyu Formation. *a, b, k*, The gathered filaments of *Gunflintia minuta* and coccoids of *Huroniospora* spp, which constitute organic-rich stromatolitic laminae. *c, d, e, h, i*, *Gunflintia minuta* Barghoorn; the arrows indicate the bulges of the filaments which are similar to the 'heterocystous' structures and are probably caused by cell degradation. *g, j*, Some coccoid microfossils. *g*, A coccoid in budding (arrowed) similar to the budding coccoid *Huroniospora* spp. from the Gunflint Iron Formation, Ontario, Canada. *j*, A coccoid morphologically comparable with *Lepitoteichos golubicii* Knoll, Barghoorn and Awramik, a coccoid taxon from the Gunflint Iron Formation of Canada. Scale bar, 10 μ m.

The discovery of a number of Precambrian microfossils over the past 20 yr has stimulated palaeontological research and raised hopes for using microfossils in studying the biostratigraphy of the Precambrian. These hopes have been tempered by the findings that some of the dominant Precambrian microorganisms show an apparent evolutionary conservatism and a considerable longevity in the fossil record. *Eoentophysalis* Hofmann¹³, for example, persists for long periods and has a modern counterpart *Entophysalis*¹⁴. Nevertheless, other Precambrian microfossils and their assemblages appear to have been limited in time^{4,15}, and the Gunflint type of microbiota seems to belong to this kind. Hofmann noted the Belcher Island microbiota is significantly different from the almost contemporaneous Gunflint microbiota, but is quite similar to the microbiota from the Bitter Springs Formation, that is roughly half its age. He concluded that the Belcher and the Gunflint microbial assemblages must have lived in different environments¹³.

The cherty dolostone beds in which the Dahongyu microbiota occur are intercalated with volcanoclastic sediments and lava, suggesting that the Dahongyu microbiota may have lived in a shallow marine environment in a region of volcanic activity. Evidence of contemporaneous volcanic activity is also found in the Gunflint Iron Formation and the Duck Creek Dolomite^{4,16}. This suggests that the Gunflint type of microbiota may have been ecologically specialized^{4,17} and may have lived in a unique environment related to volcanic activity.

The taxonomic affinity of most Gunflint microfossils has not well been defined. *Gunflintia minuta* may not necessarily be cyanobacteria, because the so-called 'heterocyst' or 'akinetes' structures may be degradational artefacts, as mentioned above. The fine filaments of *G. minuta* are morphologically similar to some modern photosynthetic and thermophilic filamentous bacteria which live and build microbial mats or stromatolites at temperatures up to 70 °C in hot springs^{17,18}. The budding coccoids of *Huroniospora* spp. with a wide range of diameter

(1–20 μm) may represent a taxonomically mixed population^{1,6,10}.

By comparing the Gunflint type microbiota from the Gunflint, Frere, Duck Creek, Tyler and Dahongyu Formations, we may conclude that: (1) *Gunflintia*–*Huroniospora* assemblage dominated a typically benthic mat-building microbial community that formed stratiform, columnar and spherical stromatolite morphotypes¹² in a particular range of environmental settings^{4,13}. (2) The Gunflint type microbiota had a worldwide distribution in the middle Precambrian. Their fossilized remains are now found in present-day North America, Australia and China. (3) These organisms, as an assemblage association, occur within a limited time span of between about 2,000 and 1,700 Myr ago.

The discovery of the Dahongyu microbiota (the oldest *in situ* microfossil assemblage in China) provides new data for general Precambrian micropalaeontology. This supports the conclusion that these microorganisms, and possibly also the environmental conditions in which they flourished, were common and geographically widely distributed, although restricted to a relatively narrow time frame in the early Proterozoic. Thus the Gunflint type microfossil assemblage may have palaeoecological as well as biostratigraphical significance.

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The myth of the mad March hare

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From literature¹, proverb² and scientific publications^{3–6}, two aspects of the behaviour of the brown hare, *Lepus capensis*, are well known. First, they 'go mad' in March. Second, boxing is their most spectacular form of male–male competition for mates. Here we show that 'madness' is no more a feature of March than of the other months of their long breeding season, and that boxing does not represent intrasexual competition but an interaction between the sexes whereby a female attempts to prevent a male from mating. Finally, we discuss why misleading statements about hare behaviour have remained unchallenged for centuries.

The brown hare is a relatively solitary herbivore, feeding mainly at night⁷. The breeding season lasts from January to August, sometimes later, and each female may produce several litters per year^{8,9}. Despite the length of the breeding season and the extensive overlap of male and female home ranges¹⁰, it is traditionally claimed that male–male competition, in the form of chasing and boxing, peaks dramatically in March^{1–6}. Indeed, scientific explanations for such activity have been offered⁶.

From 1977 a population of hares has been studied on approximately 100 hectares of the flat, permanent pastures of the

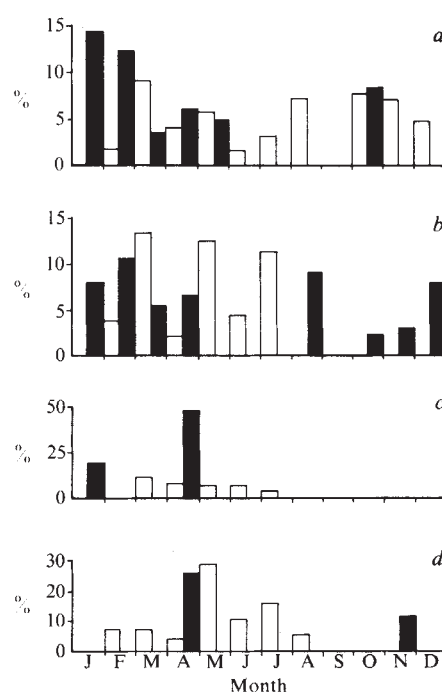


Fig. 1 The relative monthly and diel frequencies of types 1–4 (a–d, respectively) interactions throughout the year. Solid columns, sunset–sunrise, open columns, sunrise–sunset. Both diurnal and nocturnal samples have been standardized to take into account the amount of time spent observing each month. Cumulative hours of observation (1977–83) are as follows: January 112, February 123, March 158, April 167, May 177, June 181, July 271, August 120, September 32, October 96, November 67, December 63; total 1,567.

Somerset Levels, UK. Recordings were made from a vantage point 5 m above ground level. Data from over 1,500 hours of day and night-time observations by A.J.F.H. have been collected over 6 yr. From 1980 onwards, using an astronomical telescope giving magnifications of >100 times, and a catalogue of over 2,000 photographs of the hares, taken through the telescope, 10 males and 6 females have been recognized. Males and females are sexed by observation of male genitals and female teats. Individuals are recognized using features such as injury scars, facial markings and moult patterns.

The different types of competitive, reproductive behaviour which occur between individuals are defined in Table 1, together with the totals of observed encounters distinguishing occasions when all individuals involved were recognized. Figure 1 shows the monthly and diel patterns of chase (types 1–3) and boxing. Of the total number of encounters, 82.1% occurred during the usual breeding period (January–August) but only 14% of the encounters were restricted to March. Of the remaining 86%, over half (52.4%) were observed at night. Nocturnal chases occurred significantly more often during winter months (October–March) than during summer months (April–September) ($\chi^2 = 16.31$, $P < 0.001$).

Five different females and six different males were identified for the 17 boxing bouts. In every case the boxing occurred between a female and a male; no intrasexual boxing has been observed. While we do not discount the possibility that such boxing may occur, this finding is supported by an independent, unpublished study in Oxfordshire where for each of 15 bouts in which both individuals were identified, a female–male interaction was involved (W. Reade, personal communication).

We have also studied a videotape of one prolonged boxing match lasting 1 min 59 s between a female (Camelia) and a male (Cadet) filmed by Simon King in late May 1982. During this interaction (counted as one event in the analysis) there was a sequence of 35 short, rapid chases: each chase by the male of the female alternated, as the male caught up with the female, with 34 separate boxes. In 24 cases, the female alone boxed,

Table 1 Summary of the categories of interaction between hares, the numbers observed and the occasions when all participants in an interaction were identified

		Total no. of interactions recorded	Interactions in which both or all hares were sexed and individually recognized†				
			♂ → ♂	♀ → ♂	♂ → ♀	♂♂ → ♀	
Type 1 chases*:	High-speed chases between two adults which were not previously in close proximity to each other or another individual. When the hares were identified, three of four possible combinations by sex were recorded	55	8	4	2	—	
Type 2 chases:	High-speed chases between two adults, one of which at the inception of the chase was in close proximity to a third adult. Chases frequently repeated. When hares were identified, one of the males was mate guarding a female and attempted to chase off the second male	69	46	0	0	—	
Type 3 chases:	High-speed chases with more than two adults in a follow-my-leader arrangement. When hares were identified, several males were pursuing one female	8	—	—	—	4	
Type 4, boxing:	One hare lunging frontally at another with its forepaws, usually from an upright bipedal position. A boxing bout sometimes consisted of an alternating sequence of boxing and short rapid chases	25	0	♀ ↔ ♂	17	—	

* Type 1 chases were brief and not repeated and provided limited opportunity for individual recognition.

† Arrow indicates direction of chase.

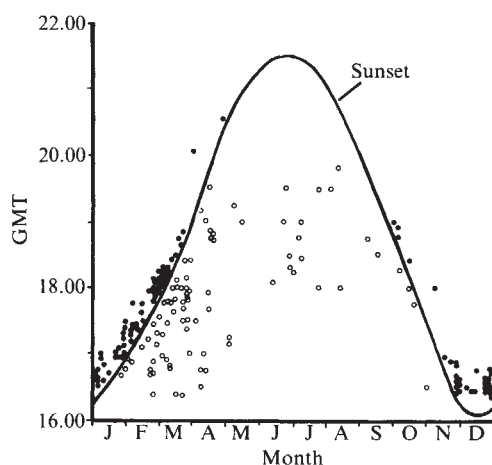


Fig. 2 Individual records (1977–83) of the times that hares were observed to leave their daytime forms in relation to the time of sunset throughout the year. This emergence from forms is equivalent to the onset of activity. ●, After sunset; ○, before sunset.

beating the head of the male with her paws as he lunged forward beneath her. In eight cases the two hares started boxing simultaneously while in the remaining two cases the male started to box after the female had initiated. In neither this case nor the other 24 boxing bouts did mating occur at the end of the encounter.

The classical view of hare 'madness' has been one of a frenzied but short period of male fighting and chasing. Indeed during the nineteenth century this episode in March was referred to as a rut². On theoretical grounds such intensive behaviours should occur in polygynous species where females are available for mating for only a short, synchronous period¹¹. The fact that the hare's breeding season is prolonged and involves several litters per female with limited reproductive synchrony should have suggested an anomaly in the behaviour of males, which would be expected to compete for mates throughout the breeding season—our results support that expectation. Clearly, much of the hare's behaviour has been overlooked. Early in the year this is probably because most interactions are nocturnal (Fig. 1):

this viewpoint is supported by Fig. 2. The fact that hares leave their resting places (forms) in the early evening throughout the year means that activity in winter starts significantly more often after sunset than in the summer ($\chi^2 = 36.3$, $P < 0.001$). In the summer, behaviour patterns are probably overlooked because the length of grass restricts an observer's view from ground level. In the present study a high observation point and flat terrain minimized that problem.

Previously, boxing was regarded as a form of male–male competition for mates. In our study all such interactions were between a female and a male. We do not yet know whether the sex difference in boxing intensity in the filmed bout is reflected in the species as a whole, but it is worth noting that females are both larger and heavier than males and in the study population males frequently have injury scars to their ears.

In view of the results presented here, we offer a tentative, short appraisal of hare breeding behaviour. Early in the year males begin to chase other males and start to search for females. A male will guard a female prior to mating and will attempt to chase away other males. If there are several males close to an oestrous female, all of them, including the guarding male, may become involved in a mating chase. Boxing is the means by which a female may prevent either a particular male from mating with her or, when not in oestrus, any male from mating. This pattern of behaviour continues throughout the long breeding season. With apologies to Lewis Carroll, the March hare is a myth.

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Spider silk as rubber

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The silks produced by spiders are exceptional structural materials. Although their tensile strengths are similar to those of cellulose, collagen and chitin, their extensibilities are considerably greater^{1,2}. Thus, the energy required to break spider silk (that is, its toughness) can be 10 times greater than for these other biological materials. The silks are crystalline proteins, with substantial portions of their polypeptide chains forming crystallites of stacked β -pleated sheets³⁻⁶. However, the extensibility of silk is attributed to non-crystalline regions, which appear amorphous on X-ray diffraction^{7,8}. The mechanical properties and macromolecular conformation of these amorphous regions have been the subject of much speculation^{1,2,7-10}, but little direct experimentation. Here we show that these regions can behave as rubber networks and exhibit rubber-like or entropy elasticity.

At room temperature ($\sim 20^\circ\text{C}$, 50% relative humidity) the silk produced by the major ampullate gland (dragline silk) of the spider *Araneus diadematus* is very stiff. If, however, an unrestrained segment is immersed in water it absorbs water and contracts to about 55% of its dry length^{9,10}. This hydration-induced contraction is accompanied by a drastic reduction in elastic modulus from $\sim 10^{10}\text{ N m}^{-2}$ to $\sim 10^7\text{ N m}^{-2}$, and a large increase in extensibility. These properties suggest that contracted silk is a protein rubber. X-ray diffraction, however, indicates that the crystalline regions are relatively unaffected by this contraction⁹. The interchain crystal dimensions are unchanged and analysis of diffraction intensities indicates that the crystals reorient but are not substantially melted. Thus, contracted silk appears to be a protein rubber filled with crystalline inclusions.

A rubber is composed of a large number of kinetically free, random polymer chains that are cross-linked to form a macroscopic network. The elasticity of such a network is due primarily to changes in the conformational entropy of the polymer chains, and only secondarily to changes in internal energy¹¹. In contrast, stiff solids such as steel, store elastic energy as changes in internal energy associated with the distortion of chemical bonds. We

have used thermoelastic measurements to determine the relative importance of internal energy and conformational entropy changes in the elasticity of contracted spider's silk. The elastic force (f) for a material deformed as a closed system at constant volume (V) and temperature (T) can be expressed in terms of changes in internal energy (E) and entropy (S), as follows¹¹:

$$f = (\partial E / \partial L)_{T,V,n} + T(\partial f / \partial T)_{L,V,n} \quad (1)$$

where L is the length and n indicates constant composition. Thus, the slope of a force-temperature plot at constant length and volume and fixed composition, $(\partial f / \partial T)_{L,V,n}$ multiplied by temperature gives the desired conformational entropy component of the elastic force (f_S), and the intercept of the force-temperature plot, $(\partial E / \partial T)_{T,V,n}$ gives the internal energy component (f_E).

In practice, it is nearly impossible to maintain constant volume and fixed composition in a thermoelastic experiment because the swelling of a sample held in equilibrium with a swelling solvent can vary with temperature. A thermoelastic relationship written for the conditions of constant pressure (P) and swelling equilibrium (eq),

$$f = (\partial H / \partial L)_{T,P,eq} + T(\partial f / \partial T)_{L,P,eq} \quad (2)$$

yields entropy and enthalpy (H) components, which contain contributions from network chain conformational changes and

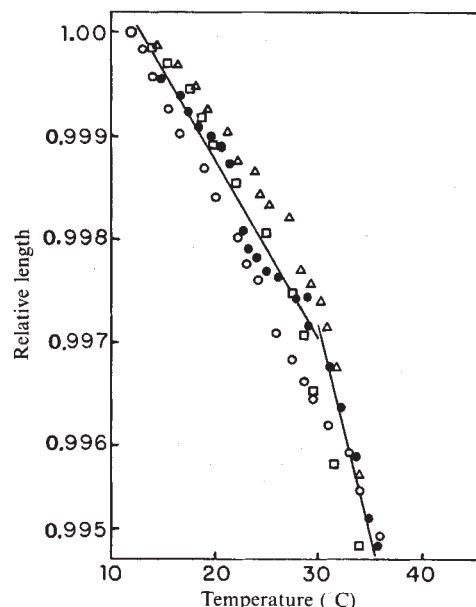


Fig. 1 Relative length versus temperature for four ~ 2.5 -cm long samples of dragline silk immersed in distilled water. The relative length appears to decrease linearly with temperature over the range 10 – 30°C , and linear regression analysis of all data up to 30°C indicates a coefficient of linear expansion of $-1.8 \times 10^{-4}^\circ\text{C}^{-1}$ ($R^2 = 0.923$, $n = 50$). Above 30°C the magnitude of the swelling change appears to increase significantly, and regression analysis of all data above 30°C yields an expansion coefficient of $-3.9 \times 10^{-4}^\circ\text{C}^{-1}$ ($R^2 = 0.855$, $n = 15$). The cause of this change in swelling behaviour at around 30°C is unknown, but to avoid problems we restricted our thermoelastic measurements to the region of linear behaviour between 10 and 30°C . Swelling in this range is assumed to be isotropic, but this assumption was not tested due to the small swelling coefficient and the small diameter of the silk fibres ($\sim 5\text{ }\mu\text{m}$).

Methods: Samples were suspended vertically in a cuvette made from microscope slides and held extended at essentially zero force by the weight of a glass rod ($\sim 5\text{ mm}$ long, 0.1 mm diameter) glued to the free end of the silk fibre. The weight of the rod caused an extension of less than 0.3% , and its effect is ignored. Changes in the length of the silk fibre were observed by following the vertical movement of its free end with a filar micrometer eyepiece on a Wild M5 dissecting microscope. The cuvette was mounted on a temperature-controlled stage and temperatures were measured ($\pm 0.5^\circ\text{C}$) with a thermistor placed near the sample.

Table 1 Enthalpy and internal energy components of elastic force of spider silk

a Sample	α	f_H/f (uncorrected)	f_E/f (corrected)	Modulus (N m^{-2})
2A	1.098	-0.36 ± 0.14	0.07 ± 0.16	9.2×10^6
1A	1.204	-0.03 ± 0.06	0.10 ± 0.07	6.2×10^6
2B	1.211	0.01 ± 0.11	0.19 ± 0.12	8.5×10^6
2C	1.293	0.08 ± 0.10	0.19 ± 0.13	1.0×10^7
Av. = 0.14 ± 0.12				
b Material		f_E/f		
Elastin		0.26		
Natural rubber		0.18		
cis-1,4-Polybutadiene		0.13		
Polydimethylsiloxane		0.19		

a, The results calculated for contracted spider's silk, as described in the text: f_H/f was calculated from equation 2, as the intercept (at 0 K) of the uncorrected stress-temperature plot, divided by the stress at 30°C (303 K). f_E/f was calculated from equation 3 using the data corrected to give stress at constant extension ratio. The error estimates were derived from standard errors calculated from the regression statistics for the stress-temperature and length-temperature data. The modulus values were calculated by dividing the stress at 30°C at each extension by the strain (strain = $\alpha - 1$), to give an indication of the stiffness of contracted silk. b, Some selected values for the internal energy component of the elastic force for natural and synthetic rubbers. (Data from ref. 13.)

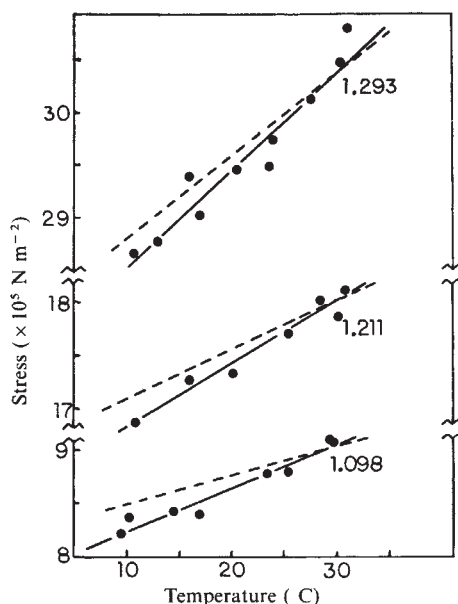


Fig. 2 Thermoelastic data for dragline silk immersed in distilled water and extended to the extension ratios indicated. Samples (~ 2 cm long) were mounted on a test frame, described previously¹⁶, in which the force is determined by following the deflection of a thin (~ 0.1 mm diameter) glass beam. The beam deflection was measured with a filar micrometer eyepiece on a Wild M5 dissecting microscope, and sample temperature was maintained and measured as described for Fig. 1. The silk sample was extended to its test length at the highest temperature ($\sim 30^\circ\text{C}$) and allowed to relax for about 1 h. The temperature was then lowered in stages to $\sim 10^\circ\text{C}$, raised in stages to $\sim 30^\circ\text{C}$ and lowered again to $\sim 10^\circ\text{C}$. The forces at each temperature were calculated from the mean of 6–10 determinations of beam deflection, and in all cases the standard error was roughly the size of the point plotted. The solid lines indicate the linear regression of the data (correlation coefficients, R^2 , are all greater than 0.91). The broken lines indicate the regression line corrected to give stress at constant extension ratio. The corrected data were used with equation 3 to calculate f_E/f .

also from polymer-solvent mixing processes. To determine f_E and f_S for contracted spider's silk we have used constant-pressure measurements, and then corrected for swelling changes by calculating the force-temperature coefficient at constant extension ratio ($\alpha = \text{extended length}/\text{initial length}$), using the following relationship¹²:

$$f_E/f = -T \left(\frac{\partial \ln(f/T)}{\partial T} \right)_{P, \alpha, eq} + T\lambda_s \quad (3)$$

where f_E/f represents the fraction of the force due to changes in internal energy, and λ_s is the coefficient of linear thermal expansion. Because this swelling correction assumes that swelling changes are isotropic, the calculated f_E/f values are reasonable approximations but are not exact.

Figure 1 shows length-temperature data for contracted silk, from which λ_s is calculated as $-1.8 \times 10^{-4} \text{ } ^\circ\text{C}^{-1}$ between 10 and 30°C . Figure 2 shows the results of thermoelastic measurements on contracted silk at three extensions. The force values are divided by the initial cross-sectional area to give a value for stress. For each extension the solid line shows the linear regression of the data; the broken line is this regression corrected for the effects of swelling to give the stress at constant extension ratio, with 30°C taken as the reference.

Table 1 gives the enthalpy (f_H/f ; equation 2) and internal energy (f_E/f ; equation 3) components of the elastic force calculated from our data. The enthalpy (that is uncorrected) component of the elastic force is negative at low extensions and becomes increasingly positive as extension increases. It is unlikely that the amorphous chains crystallize with extension because the enthalpy component, which would be negative for such a strain-induced crystallization, becomes positive as extension increases. Rather, the negative enthalpy component

at low extensions arises from the thermal-swelling behaviour of the material. When the data are corrected for swelling effects, the internal energy component (f_E/f) becomes a small and essentially constant, positive fraction of the elastic force. The average f_E/f value of 0.14 is similar to that seen for other rubbery materials (Table 1b)¹³, and indicates that roughly 85% of the retractive force of contracted spider's silk is due to changes in polymer chain conformational entropy.

The thermodynamic properties of contracted dragline silk indicate that the material is a rubber. Apparently, when immersed in water the protein chains in the amorphous regions have sufficient kinetic freedom so that bulk deformations do not cause any major changes in bond energy potentials. Rather, energy is stored primarily as a change in the conformational entropy of these chains. The crystalline regions presumably serve to cross-link the amorphous chains into a network. This proposed structure for contracted (that is, hydrated) silk may shed important light on the behaviour of the silk in its normal, non-contracted state. Protein rubbers in general are rubbery only when swollen in water or some other polar solvent¹⁴. A lowered water content decreases kinetic freedom of the peptide chains, and the material enters its glass transition, where stiffness increases dramatically, extensibility is reduced, and viscoelastic energy dissipation becomes very important¹⁴. We propose that the dragline silk of *A. diadematus* in room conditions should be thought of as a rubber that functions near the middle of its glass transition. The amorphous regions provide a reasonably stiff, energy-absorbing matrix within which are embedded very stiff, crystalline inclusions. In this respect silk, like nylon, is a fibre-reinforced composite, and the analysis of silks as composite materials may well explain their unusually high breaking energies¹⁵.

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Inability of Rous sarcoma virus to cause sarcomas in the avian embryo

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The injection of Rous sarcoma virus (RSV) into the wing web of newly hatched chicks causes a rapidly growing sarcomatous tumour which is palpable within 1 week of inoculation¹; and cultures of fibroblasts derived from chick embryos (CEF) and infected with RSV become rapidly transformed². Genetic studies have determined that expression of a single viral gene, designated *v-src*, is necessary for neoplastic transformation³. This gene codes for a 60,000-molecular weight phosphoprotein termed pp60^{src}, which

possesses a protein kinase activity that phosphorylates polypeptides on tyrosine residues and is constitutively expressed in infected CEF cells⁴. It has been suggested that transformation, and possibly tumorigenesis, may result solely from the consequences of this increase in tyrosine phosphorylations⁴. The pathogenicity of RSV in chick embryos *in ovo* is less clear. Murphy and Rous⁵ suggested that RSV may have caused tumours in "various tissues" of "some embryos", but the subsequent studies of Milford and Duran-Reynals⁶, as well as several other laboratories^{7,8}, failed to find any evidence of intraembryonic tumours in RSV-infected early embryos. The findings of Duran-Reynals, if correct, cannot be explained easily in view of our present understanding of RSV tumorigenicity. Thus, we have re-examined the interaction of RSV with the avian embryo and confirm here that RSV is non-tumorigenic and non-teratogenic when microinjected into day 4 chicken embryos. In addition, we found that (1) the virus not only replicates in the embryo, but it also expresses an active *src*-specific protein kinase and (2) once the cells from the infected limbs are disrupted and placed in culture, they are capable of expressing the transformed phenotype after a 24-h delay.

Using a capillary tube pulled to 40–75 μ m diameter, we inoculated the limb bud of day 4 chicken embryos (stage 22–24)⁹ with 10^4 – 10^5 focus-forming units of Prague-A (PRA) or Schmidt-Ruppin-D (SRD) RSV. Control embryos were inoculated with virus-free medium (medium 199 supplemented with 2% tryptose phosphate broth, 2% fetal calf serum and 1% chick serum—designated medium 2-2-1). Embryos were allowed to continue *in ovo* and eggs were candled daily. Some embryos were killed and analysed at various developmental stages; others were examined at death. We found no appreciable difference between the survival of control and virus-inoculated embryos until 7 days post-injection when the mortality of virus-inoculated embryos increased relative to the inoculated controls, suggesting that events specific to the virus infection had subsequently begun to manifest themselves (data not shown).

To date we have inoculated over 1,500 embryos. Those examined for tissue histology and gross morphology at various times during development showed no indication of neoplastic growth or teratogenesis. Figure 1*d, e* shows an example of a limb bud from a 14-day embryo which had been inoculated with virus on day 4. By morphological criteria, chondrogenic and myogenic differentiation are unimpaired. A section through the limb bud of an embryo inoculated with medium alone is presented for comparison (Fig. 1*a*). 25% of the RSV-containing embryos developed blood blisters that varied in size from 1 to 10 mm and resembled the non-neoplastic haemorrhagic lesions first described by Duran-Reynals^{6,10} (Fig. 1*b, c*). No lesions were observed in control inoculated embryos, nor in embryos infected with transformation-defective (*td*) RSV (not shown).

The absence of response to RSV in embryonic limb buds contrasted sharply with the response in the wing of newborn chicks. As demonstrated by others^{1,11}, we found that, within 10 days, 75% of the newborn chicks inoculated with 10^7 FFU of RSV has palpable tumours, >5 mm in diameter. Furthermore, as few as 10^4 FFU generated tumours in 15% of the birds within the same time frame. The histological appearance of haemorrhagic lesions was easily distinguishable from that of RSV-generated tumour tissue taken from a 10-day-old chick inoculated 24 h after hatching (compare Fig. 1*b* and *c* with *f*). The tumour tissue was invasive and contained a heterogeneous population of cells with large irregular nuclei characteristic of neoplastic tissue. As we used cloned viruses and specific pathogen-free (SPF) eggs, we have essentially ruled out artefacts of possible interference by endogenous or exogenous viral contaminants. The results confirm those of Duran-Reynals and others^{6,7,10}, and suggest that early embryos are indeed refractory to the tumorigenic potential of RSV.

To determine whether or not RSV replicates *in ovo*, control and infected embryos were killed on day 14, and various tissues were homogenized and screened for the presence of reverse transcriptase¹². We found more than a 10-fold increase in activity of the enzyme in the inoculated limb tissues, and a significant

increase of activity in tissues distal to the infected limb bud (Table 1*a*). Virus from these embryos formed up to 2×10^5 FFU per μ g of tissue DNA. Tissue isolated from 10-day-old tumours in young chicks contained 0.5 – 2×10^6 FFU per μ g of DNA. It should be pointed out that as we neither observed an obvious tumour nor assayed the entire wing, some cells may not be infected. On the other hand, tissue taken from the tumours in the newborn, by definition, was viraemic. Some virus-inoculated embryos did not show the presence of virus when screened at day 14; we presume that, in these embryos, the injection was unsuccessful.

Despite the absence of tumours, tissues from infected embryos contained elevated levels of viral kinase activity, at times comparable with the levels found in tumour tissue. The level of kinase activity varied among embryos, but in the inoculated limbs there were increases as high as 50-fold compared with control tissues. Other tissues also contained elevated levels of kinase activity, suggesting that the infection had spread throughout the embryo. Elevation of kinase activity was detectable as early as 2 days after inoculation; therefore, full viral gene expression probably occurred soon after infection.

Table 1 Parameters associated with virus production in RSV-infected embryos

<i>a</i> Reverse transcriptase activity			
Tissue	Fold increase		
Plasma	2.4		
Wing	10.1		
Breast	3.0		
Viscera	1.6		
Brain	2.1		

<i>b</i> Infectious virus and kinase activity			
	Uninfected	Infected	Tumour tissue
FFU $\times 10^{-5}$ per μ g DNA	0	1.3 $\pm$ 0.8	11.3 $\pm$ 5.7
Kinase per μ g DNA	7.5 $\pm$ 2.1	190 $\pm$ 83	282 $\pm$ 57

<i>c</i> Distribution of kinase activity	
Tissue	Fold increase
Infected wing	51.00
Liver	2.7
Opposed wing	3.7

Embryos were inoculated as described in the text, killed on day 14 and tissues were immediately frozen in a dry ice bath. *a*, Tissues were subsequently homogenized in 1.5 ml of 10 mM Tris, 1 mM EDTA. 0.05 ml of each tissue specimen was assayed for reverse transcriptase activity according to the procedure of Tereba and Murti¹², and 0.1 ml was assayed for DNA content by the method of Hinegardner⁴². Fold increase is the ratio of reverse transcriptase activity (normalized to DNA) in the tissues of virus-inoculated embryos compared with the normalized reverse transcriptase activity in the corresponding tissue of controls. *b*, Tissues were prepared as in *a* except that, for kinase analysis, they were homogenized in disruption buffer (25 mM HEPES buffer (pH 6.8), 1% Nonidet P-40, 0.5 M NaCl, 5 mM EDTA and 2% Trasylol). Kinase assays were done as described⁴³ except that the reaction buffer was 10 mM HEPES, 5 mM MnCl₂, 16 mM NaCl. Each sample was also assayed for DNA content as described⁴². Focus-forming activity was determined as described elsewhere⁴⁴ and normalized to the DNA content of the same tissue. Kinase activity was quantified by solubilizing the IgG band, counting the radioactivity and normalizing the counts to the DNA content of the sample. *c*, Fold increase refers to the ratio of kinase activity in tissues infected with virus and normalized to DNA content, compared with the corresponding normalized tissue in controls. Note that despite a 10-fold reduction in FFU, kinase activity at times is as high as in the tumours. As the infected wing cells *in ovo* have 'normal' morphology, they may not be able to replicate the virus at the same rate as transformed cells. This is supported by our preliminary data which indicate that transformed cells plated on normal extracellular matrix flatten out and, while they contain appreciable kinase activity, virus replication is reduced considerably⁴⁵.

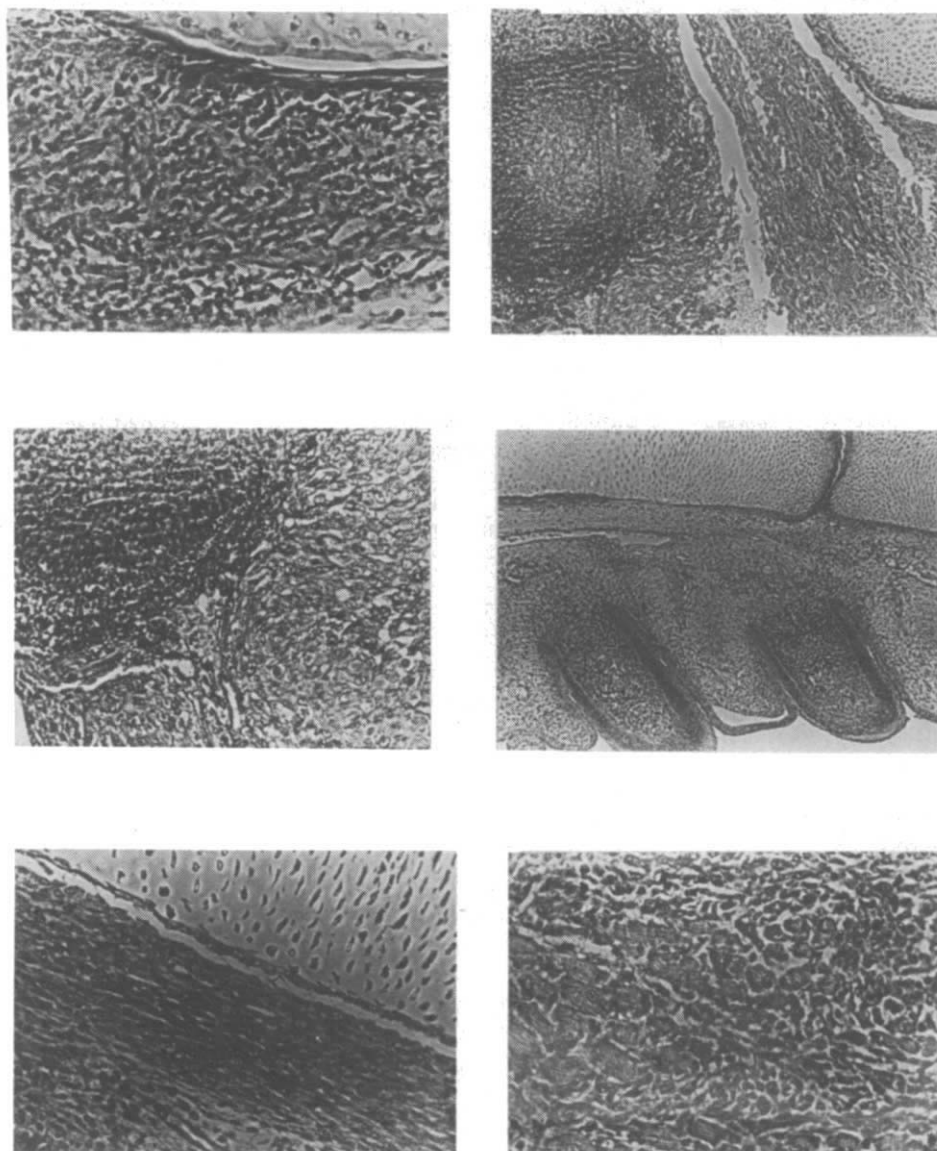


Fig. 1 Histology of inoculated limb buds and tumour tissue. *a–e*, Embryos were inoculated on day 4 as described in the text and were killed on the indicated day. The inoculated limb was removed and fixed in Bouins, embedded in paraffin and sectioned (5 μ m). Sections were photographed after staining with haematoxylin and eosin using a Nikon diaphot microscope. *a*, Control limb inoculated with medium alone and killed on day 13. $\times 280$. *b*, Limb inoculated with 10^5 FFU of SRD RSV and killed on day 13. $\times 105$. Note the haemorrhagic lesion on the left. *c*, Same as *b*. Lesion is in the upper left quadrant. $\times 280$. *d*, Limb inoculated with 10^5 FFU of SRD RSV and killed on day 12. $\times 70$. No abnormal morphology was noted. *e*, Same as *d* showing the perichondrium and adjoining tissues. $\times 280$. *f*, Tumour tissue from 10-day-old chicken, inoculated with 10^5 FFU of SRD RSV after hatching. Tumours first appeared on day 7. $\times 280$.

Embryos which failed to show an increase in kinase activity also failed to show reverse transcriptase or focus-forming activity, and probably had not become infected, as discussed above.

We next addressed the following questions: (1) How do the cells from the limbs of infected 14-day-old embryos behave after being disrupted and placed in culture? (2) Are the cultured cells from the limb buds of 4-day-old embryos as susceptible to transformation and virus production as cells from older embryos? Using morphology and the rate of sugar uptake as two commonly measured criteria of transformation, we compared cultures prepared from the wing of inoculated embryos with either cultures derived from the wing of control embryos which were infected at the time of plating, or cultures derived from tumours generated in newly hatched chicks (Fig. 2). Cells were cultured in 25-mm multiwell plates at high density ($4\text{--}5 \times 10^5$ cells cm^{-2}) to discourage the horizontal spread of infectious virus. Chick cells cultured in these conditions grow slowly; the percentage of cells infected in the first 72 h is reduced by a factor of 5 or more compared with cultures infected at low density (data not shown). Despite high kinase activity which was elevated in the intact tissue and which remained high after disruption, cells infected *in ovo* appeared quite normal initially, by the criteria of both morphology and rate of sugar uptake. However, within 24 h the cultures became morphologically

transformed (*en masse* rather than as foci) and the rate of sugar transport increased. This 'delayed' transformation is not due to the process of tissue disruption associated with the preparation of cultures, as cultures derived from tumour tissue or from fully transformed cultures retained a transformed morphology and high sugar uptake immediately after trypsinization and replating. On the other hand, cultures derived from normal embryos which were infected after plating (multiplicity of infection (MOI) = 0.2; approximately the same virus-to-cell ratio found in the infected limb tissue at the time of culturing) began to show foci of transformation and a stimulation of the rate of sugar transport only after an interval of 72 h.

Thus, embryonic cells infected *in ovo* retain their normal phenotype *in ovo*, as well as initially after plating, but the ability to display a transformed phenotype is rapidly acquired after the cells are placed in culture. It is possible that either the culture conditions are permissive for the expression of the transformed phenotype, or alternatively, these cells may experience an acceleration of the developmental programme which would normally lead to the tumour permissiveness observable in newly hatched chicks. The notion that the developmental programme of chick cells may accelerate in culture at high densities has been suggested by previous work in this laboratory¹³.

To determine whether cells from the limb bud of day 4 embryos are as susceptible to transformation, and whether they

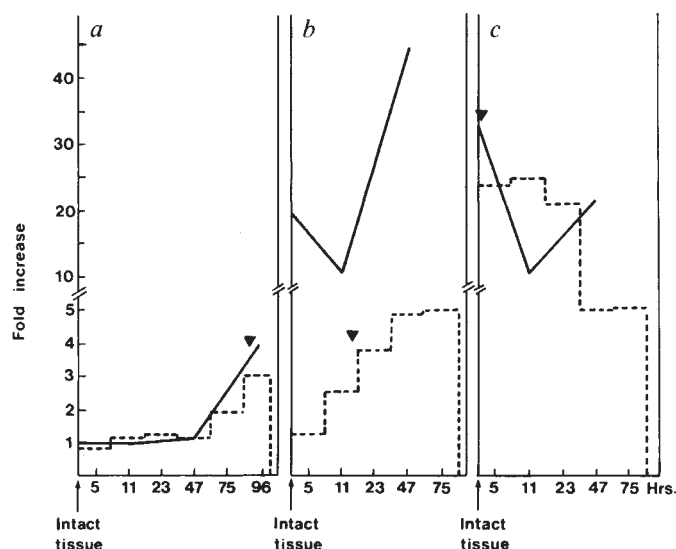


Fig. 2 Determination of transformation in cultures derived from the wing of control embryos infected at the time of plating (a), cultures prepared from the wing of inoculated embryos (b), and cultures derived from tumours generated in newly hatched chicks (c). Embryos were inoculated and killed as described in the text. The inoculated limb was removed and a portion of intact tissue was assayed for kinase activity as described in Table 1. The rest was digested for 45 min at 39 °C in a solution of medium F-10, 0.15% trypsin, 0.3% collagenase (type II) and 5% fetal calf serum. An aliquot was checked under a microscope to determine the extent of digestion and the incubation time was adjusted accordingly. After digestion, the cells were washed twice in medium 2-2-1, and plated at high density ($4 \times 10^5 \text{ cm}^{-2}$). At the times indicated, 2-deoxyglucose⁴⁶ (broken lines) and kinase⁴³ (solid lines) assays were performed. The assays for each time point were normalized to that of cultures of cells derived from uninfected embryos and are expressed as the fold increase relative to the control. ▼ Indicates the time of appearance of morphological transformation.

produce progeny virus at the same rate, as cells derived from the limbs of day 14 embryos, limb bud tissues from the respective embryos were dissociated, plated at low density and infected with RSV at a high multiplicity of infection (MOI = 2). Cultures were subsequently screened for reverse transcriptase activity, sugar uptake and morphology. Predictably, the growth rates and subsequent morphologies of infected and uninfected cultures derived from day 4 embryos differed from those of their day 14 counterparts. However, the increase in sugar uptake and the production of viral particles (per cell) were comparable in both infected cultures (Fig. 3).

The infection and replication of RSV in certain avian embryo cell types in culture in the absence of transformation has been reported^{14,15}. The infected limb cells in our experiments differ somewhat in that they express their transformed phenotype once placed in culture. This is not a selection phenomenon as we have shown recently that 90% of the isolated limb cells are positive for the presence of viral antigens before culturing (D.S.D., M. Gates and M.J.B., unpublished); and as shown in Fig. 2b, transformation in these cultures occurs *en masse* within 24 h. Dunkel and Groupe¹⁶ found, several years ago, that embryonic day 3 limb buds infected *in vitro* and grafted onto the chorioallantoic membrane of 9-day-old eggs, show virus replication long before tumour foci appear. The eventual presence of tumours may be due to the disruptive nature of the grafting procedure, the altered developmental environment, or other differences in experimental conditions.

We are presently considering three general hypotheses which might explain the apparent inability of RSV to cause sarcomas in chick embryos. First, the intracellular concentration of viral kinase activity (or its localization) may be suboptimal. Second, a cellular component, required for tumorigenic expression, may

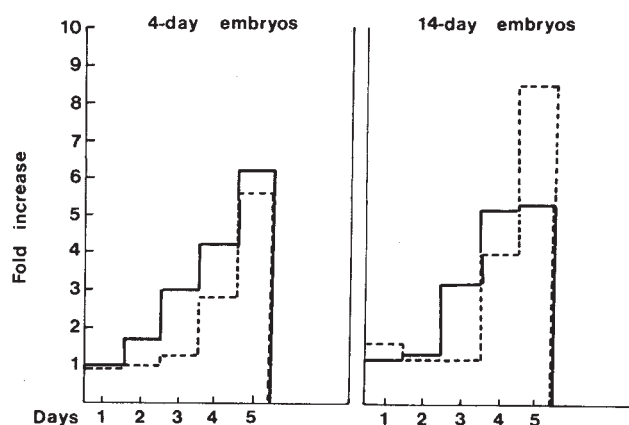


Fig. 3 Susceptibility to transformation of cells from limb buds of day 4 and day 14 embryos. The limb buds from several 4-day-old embryos were microdissected, pooled and dissociated as described in Fig. 2 legend. The lower limb of a 14-day-old embryo was prepared in the same fashion. Both were plated at low density ($5 \times 10^4 \text{ cells cm}^{-2}$), and half the plates from each group were infected with SRD RSV at a MOI of 2.0. 2-Deoxyglucose uptake (broken lines) and reverse transcriptase activity (solid lines), as well as morphology and cell number, were monitored daily. Each of the infected cultures was normalized to its uninfected counterparts and the result is expressed as the fold increase compared with the normal control.

be absent from the early embryo; this component might be a target for the viral kinase¹⁷⁻²⁰, for an 'additional' *src* gene-mediated activity²¹⁻²⁶, or a hormone, growth factor or endogenous promoter²⁷⁻³⁰. In addition to published data^{24,25} for cases in which kinase activity alone does not appear to be sufficient for transformation in culture, there are recent data to indicate that some temperature-sensitive mutants of RSV have comparable kinase activity at both permissive and non-permissive temperatures (A. Stoker, P. Enrietto and J. Wyke, personal communication). This indicates the possible importance of other activities and/or domains in *pp60^{src}* for transformation²¹⁻²⁴. Third, the early embryo may contain inhibitors for any of these necessary interactions^{31,32}. We are presently determining whether or not known targets of *pp60^{src}* are phosphorylated in tyrosine *in ovo*.

We have been unable to determine whether embryos infected on day 4 of development will ultimately develop tumours. Due to the lethality of the virus around days 12-15, we have hatched only four of these embryos: none contained detectable tumours, although all four died within 4 days of hatching from as yet undetermined causes. Several examples exist both for sustained suppression of the neoplastic potential^{31,33,34} and for temporary suppression related to the developmental stage of the animal^{16,35-39}, but obviously more embryos must be hatched to draw any firm conclusions.

How general are the implications of these findings for retroviral tumorigenicity in the embryo? Clearly, there is target specificity, and the age of the embryo and possibly route of injection are important factors. Smith and Iranyi⁴⁰ found evidence for target 'stem' cells in 12-day-old embryos when injected intravenously with myeloblastosis-associated virus (MAV)-2(0). These chickens developed suppression of bursal and thymic lymphoid cell development 2-3 weeks after hatching. A mutant of avian erythroblastosis virus appears to produce erythroblastosis in 12-day-old embryos efficiently, but it does not produce sarcomas in the embryos. Nevertheless, sarcomas are readily produced in 1-week-old chicks by the same mutant⁴¹. This latter finding supports our general conclusion that production of sarcomas in the embryo is either suppressed or requires additional factors not present in the embryo.

Note that most of the studies with chick embryos have been done using embryos over 10 days old and our studies were done

with 4-day-old embryos. It is possible that the susceptibility of the embryo to RSV tumorigenicity may increase as the embryo develops. It is also possible that if factors from transformed cells were present, one could induce tumours even in 4-day-old embryos. In preliminary studies we have failed to induce tumours with transformed cells. However, we could not ascertain whether or not the cells remained viable and will in future use cells such as quail that could be distinguished from chick cells.

It seems that while kinase activity of pp60^{src} may be necessary for RSV-mediated transformation and tumorigenesis, there are one or several other developmentally related criteria which must be met before expression of the malignant phenotype is possible. The temporal sequence of the expression of viral and cellular factors may also be an important determinant. Infected cells, once placed in culture, overcome the *in ovo* restriction and become transformed rapidly. The mechanisms of both the restriction *in ovo* and transformation in culture are being investigated.

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A relationship between the yeast cell cycle genes *CDC4* and *CDC36* and the *ets* sequence of oncogenic virus E26

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We report here significant primary sequence homology among the predicted translational products of three genes: *CDC4*, *CDC36* and *ets*. *CDC4* and *CDC36* are *Saccharomyces cerevisiae* cell division cycle genes, while *ets* is a transformation-specific sequence of avian erythroblastosis virus E26. The deduced primary structures of the three gene products were compared by computer to a large data base of known and predicted protein sequences¹. The search revealed 22.0-25.5% identity over regions of 140-206 codons, respectively between the different pairwise combinations. For these particular sequences, these identity scores fall 3.4-4.0 standard deviations above the empirically-determined mean values of fortuitous similarity. *S. cerevisiae* calls require *CDC36* and *CDC4* in order to complete two early events in the cell cycle: execution of start (*CDC36*)² and spindle pole body separation (*CDC4*)³. In virus E26, the *ets* sequence is linked in frame with Δ gag and myb^E in the tripartite structure 5'- Δ gag-myb^E-*ets*-3', comprising the E26 transforming oncogene^{4,5}. The homologies described here suggest that the biochemical functions or regulation of the *CDC4*, *CDC36* and *ets* products may be related.

Figure 1 shows an alignment of the inferred amino acid sequences of the entire *CDC36* translational product (middle line) with homologous regions of the *CDC4* and *ets* products. The locations of these sequences in their respective genes are shown in Fig. 2. The lengths of the *CDC4*, *CDC36* and *ets* products are 779, 191 and 491 amino acid residues, respectively. Above the complete 191-residue *CDC36* sequence in Fig. 1 is the 149-residue C-terminal portion of the *CDC4* product. Below *CDC36* is a 206-residue region from the middle of the *ets* product (Fig. 2). The most striking similarities are observed near the N-terminus of the *CDC36* product. In this region, between *CDC36* Ala 8 and Ser 22, 6 out of 15 residues match among all three sequences, and 7 out of 15 match between two sequences. Another region of substantial similarity is the 10-residue region from *CDC36* Tyr 39 to Gln 48, in which the *CDC36* product

Table 1 Homology between pairwise combinations of *CDC4*, *CDC36* and *ets*

Gene pair	% Identity	Standard deviations above mean	Probability of fortuitous match
<i>CDC4</i> - <i>CDC36</i>	25.5	4.0	0.003%
<i>CDC36</i> - <i>ets</i>	22.0	4.0	0.003%
<i>ets</i> - <i>CDC4</i>	22.8	3.4	0.033%

% Identity refers to per cent of matching residues per sequence length. Standard deviations above mean refers to the number of standard deviations the alignment scores fall above a mean value of chance homology empirically determined by aligning a large number of scrambled sequences of identical composition and length (ref. 1 and see text). Probability of fortuitous match refers to expected per cent of random sequences with alignment scores greater than or equal to that of the genuine gene pair alignment, based on an empirical distribution of alignment scores of scrambled sequences (ref. 1 and see text).

Fig. 1 Alignment of the predicted primary structures of the *CDC4*, *CDC36* and *ets* products. Amino acid identities are boxed; conservative changes are enclosed by dashed boxes. Sequence gaps necessary to obtain optimal alignment are represented by spaces. The coordinates refer to the *CDC36* residue number, with the initiating Met=1. The *CDC36* product is aligned for maximal homology with the *CDC4* and *ets* products. This alignment does not give the maximal homology between *CDC4* and *ets*. Single-letter abbreviations for the amino acid residues are as follows: A, alanine; C, cysteine; D, aspartic acid; E, glutamic acid; F, phenylalanine; G, glycine; H, histidine; I, isoleucine; K, lysine; L, leucine; M, methionine; N, asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; W, tryptophan; Y, tyrosine.

Methods: *CDC36* and *CDC4* were isolated on recombinant plasmids by transformational complementation (ref. 18 and unpublished data). Each was shown to represent the authentic *CDC36* and *CDC4* genes by their ability to recombine at their respective genomic loci (ref. 18 and unpublished data). The *CDC36* and *CDC4* genes were localized by subcloning, RNA blot analysis and R-loop mapping (ref. 18 and unpublished data). *CDC4* was sequenced by chemical degradation methods¹⁹ and found to contain a large open reading frame of 779 codons. The 149 C-terminal residues of the *CDC4* predicted translational product are shown. Sequencing of *CDC36* by the ml3-dideoxy method²⁰ revealed an open reading frame of 191 codons within the 615-base pair transcribed sequence. The complete *CDC36* deduced translational product is shown. A clone prepared from cDNA transcribed *in vitro* from E26 genomic RNA was sequenced by the ml3-dideoxy method²⁰ and found to contain an E26 transformation-specific (*ets*) sequence flanked by *myb*^E (an internal 0.8-kb subset of the 1.2-kb AMV-related sequence, *myb*^A) and an *env*-related sequence⁴. *ets* encodes the C-terminal 491 amino acid residues of p135 (ref. 4 and Fig. 2). 206 residues of the *ets* predicted translational product are shown.

matches either the *CDC4* or *ets* products at 8 of the 10 positions. We view these similarities as strong evidence that these gene products are related. Overall, the homology between the different pairwise combinations ranges from 22.0 to 25.5%. These scores were compared with the results of a 'jumble test', in which sets of scrambled sequences whose compositions and lengths are identical to those of the two sequences under study (that is, between 149, 191 and 206 codons of *CDC4*, *CDC36* and *ets*, respectively; see Fig. 2) are subjected to the same alignment and scoring procedure used for the authentic alignment¹. The homology values of the genuine sequences were found to lie 3.4–4.0 standard deviations above the mean values of the randomized sequences, indicating a low probability that the similarities detected are fortuitous (Table 1). We cannot rigorously exclude the possibility that any of the similarities detected here are the results of convergent, rather than divergent, evolution. However, since a particular protein structure can be obtained from many different amino acid sequences, sequence convergence extending over significant lengths (for example, >20 residues) is highly unlikely. Additionally, the requirement for both the *CDC4* and *CDC36* products in the G₁ phase in the same organism (see below) suggests an ancestral relationship for these two genes.

CDC4 and *CDC36* both define early events in the cell cycle of the budding yeast *S. cerevisiae* (see ref. 6 for review). Cells defective in *CDC36* arrest division at start, a point in the late G₁ phase of the cell cycle^{2,7}. Start appears to be the cell cycle stage at which cell division is normally controlled, as wild-type cells deprived of essential nutrients or responding to mating pheromone also arrest at start⁷. Cells arrested at this stage are unbudded and have not yet undergone duplication of the spindle pole body, which serves as the microtubule-organizing structure³. The next defined step in the mitotic cycle requires *CDC4*. Cells arrested by the *cdc4* defect also fail to initiate DNA synthesis⁸ and have duplicated but unseparated spindle pole bodies³. Completion of the *cdc4*-sensitive step results in separation of the duplicated spindle pole bodies, which migrate to opposite sides of the nucleus to become the poles of the mitotic spindle³. Although arrest by *cdc4* is morphologically advanced with respect to start, cells at both stages remain in G₁. Moreover, both of these arrests share the properties that (1) entry into the S phase requires additional protein synthesis⁹ and (2) the cells

may enter directly into meiosis without completing a mitotic cycle if transferred to the appropriate medium¹⁰. By contrast, *CDC7* defines a later period of the G₁ phase which lacks these properties. Therefore, it is not unreasonable to suppose that the structural feature shared by the products of *CDC4* and *CDC36* is essential to their function or regulation in a segment of G₁.

ets is a genomic sequence of the avian erythroblastosis virus E26^{4,5}. E26 is related to avian myeloblastosis virus (AMV) in that both viruses are replication-defective, contain a *myb* (or *amv*) sequence, and cause acute myeloblastic leukaemia yet do not transform chicken fibroblasts in culture^{11,12}. However, the predominant disease caused by E26 is erythroblastosis, whereas AMV causes myeloblastosis exclusively^{13,14}. The E26 oncogene is a tripartite hybrid composed of the in-frame fusions 5'- Δgag -*myb*^E-*ets*-3' (refs 4,5). The Δgag , *myb*^E and *ets* sequences comprise 0.8, 0.8 and 1.5 kilobases (kb), respectively, of the hybrid gene⁴. This oncogene codes for a putative transforming protein of 135,000 molecular weight, termed p135 (ref. 12), of which *ets* encodes the C-terminal 491 residues⁴ (Fig. 2). DNA hybridization experiments revealed the existence of cellular proto-*ets* sequences, which are unlinked to proto-*myb*^{4,5}.

The biological functions of the *CDC4*, *CDC36* and *ets* products remain unknown. The product of another yeast start gene, *CDC28*, is homologous to the products of the *src* gene family, many of which have been shown to encode protein kinases¹⁵. In view of these results it is conceivable that the products of retroviral transforming genes share functional domains with proteins that control cell division. Other authors have reported

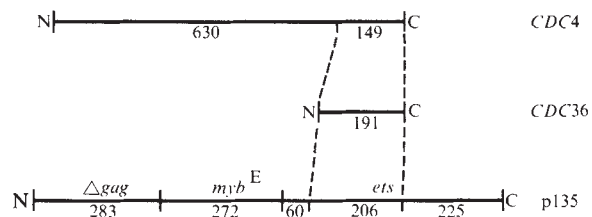


Fig. 2 The locations of the sequences compared in Fig. 1 relative to the complete gene products. Numbers below each line indicate the residue length of that section. Dashed lines enclose homologous sequences presented in Fig. 1. N and C refer to the amino and carboxy termini, respectively. Drawn approximately to scale.

significant homology between other yeast sequences and the vertebrate oncogenic sequence *ras*^{16,17}. Our findings extend these observations and support the notion that the mechanisms controlling cell division in yeast and cellular proliferation in vertebrates may be related.

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A novel protease from yeast with specificity towards paired basic residues

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Paired basic residues have been observed as sites of proteolytic processing of prohormones in a wide range of eukaryotic species^{1–3}. This strongly suggests that proteases exhibiting specificity towards paired basic residues may be involved in prohormone processing, but candidate enzymes have not so far been identified. Yeast *Saccharomyces cerevisiae* α -cells synthesize and secrete α -mating factor, a peptide of 13 amino acids^{4,5}, the processing of which from a larger precursor involves cleavage at paired basic residues (–Lys–Arg–)^{3,6}. We have therefore used them as a simple model system for the study of prohormone processing and report here the identification, in cell lysates, of a novel protease which specifically recognizes and cleaves the peptide bonds between consecutive basic residues. The purified enzyme, which we have called propheromone-convertase Y, has a molecular weight (MW) of around 43,000. It cleaves various peptide substrates at paired basic residues, but not at single basic residues, implying it is distinct from trypsin-like proteases. Its unique substrate specificity suggests the enzyme may be involved in propheromone processing *in vivo*.

Proteolytic activity specific to consecutive basic residues was monitored in lysates of yeast *S. cerevisiae* α -cells by measuring in a sensitive radioimmunoassay⁷ the amount of Tyr–Gly–Gly–Phe–Leu–Arg ([Leu]enkephalin–Arg) released from the synthetic C-terminally α -amidated peptide Tyr–Gly–Gly–Phe–Leu–Arg–Arg–Phe–Ala–NH₂ ([Leu]enkephalin–Arg–Arg–Phe–Ala–NH₂). Since the antiserum used in the radioimmunoassay was directed against the C-terminus of [Leu]enkephalin–Arg, neither the substrate nor its fragmented peptides other than [Leu]enkephalin–Arg showed immunoreactivity⁷.

The soluble fraction of the yeast lysates was chromatographed on a column of DEAE–Sephadex A-25. The enzyme activity

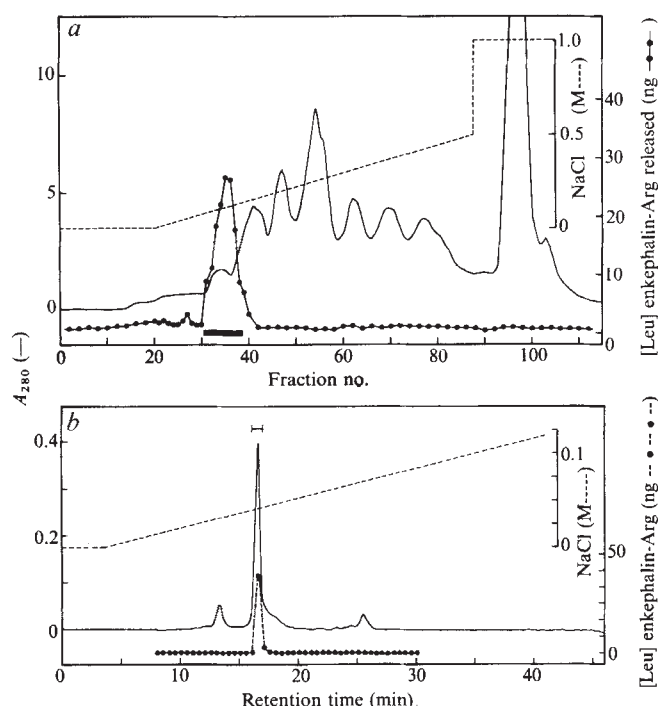
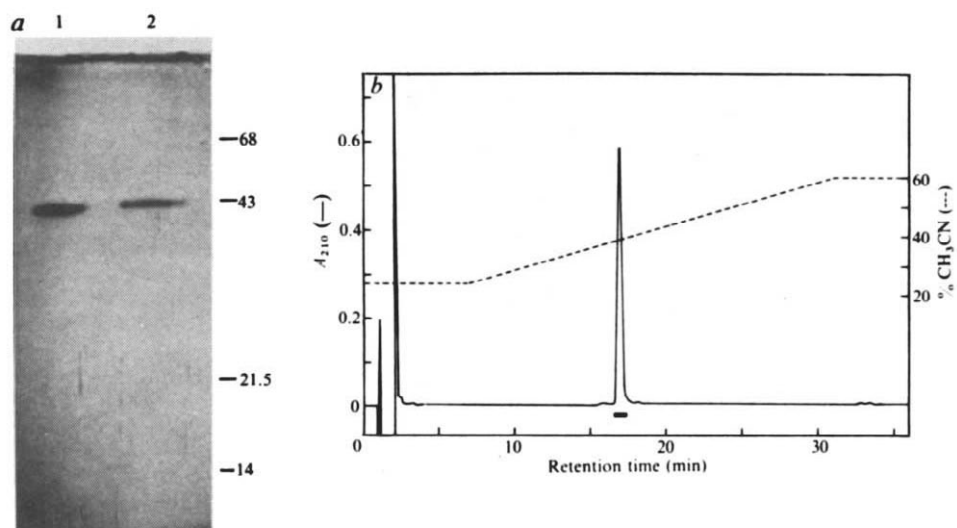


Fig. 1 Purification of the enzyme from lysates of yeast α -cells. *a*, DEAE–Sephadex A-25 chromatography of crude extracts of yeast α -cells. *b*, Final purification of the enzyme by second FPLC on a Mono Q anion-exchange column.

Methods: Haploid strain *S. cerevisiae* X-2180 1B (α -mating type cell) was supplied by Dr T. Tanaka, Santory Ltd. For purification of the enzyme, cells were grown aerobically at 28 °C for 50 h in 6 l of YM-medium, containing 0.3% yeast extract, 0.3% malt extract, 0.5% polypeptone (pancreatic digest of casein) and 1% glucose. Cells (wet weight 45 g) were lysed by incubation with Zymolyase-60,000 (Seikagaku Kogyo) at 35 °C for 4 h (ref. 17), and homogenized with a Polytron homogenizer. After centrifugation at 30,000g for 30 min, ammonium sulphate was directly added to the supernatant to a final concentration of 90% saturation and the resulting precipitate was resuspended in 10 mM Tris–HCl buffer, pH 8.0, and dialysed against the same buffer. The dialysate was applied to a column of DEAE–Sephadex A-25 (3 × 30 cm), equilibrated with the same buffer. The column was washed with 10 mM Tris–HCl buffer (pH 8.0) and then eluted with a 1-l linear gradient from 0 to 0.5 M NaCl in the same buffer. Fractions of 15 ml were collected at a flow rate of 30 ml h^{–1}. Enzyme activity was assayed by using the synthetic enkephalin-containing peptide, Tyr–Gly–Gly–Phe–Leu–Arg–Arg–Phe–Ala–NH₂, as a substrate. Aliquots (20 μ l) from each fraction were incubated at 37 °C for 2 h with 0.1 nmol of the substrate in 100 μ l of 0.1 M ammonium acetate buffer (pH 5.5) containing 1 mM EDTA and 1 mM dithiothreitol. After stopping the reaction by 10 min of boiling, the amount of [Leu]enkephalin–Arg generated was measured by radioimmunoassay using an antiserum directed against the C-terminus of [Leu]enkephalin–Arg as reported previously⁷ (—●—●—●—). The enzyme fractions (marked with bar) obtained from DEAE–Sephadex A-25 chromatography were pooled and concentrated by 90% ammonium sulphate precipitation. The precipitate was dissolved in 8 ml of 0.1 M ammonium acetate buffer (pH 5.5) containing 1 mM EDTA and applied to a column (3 × 140 cm) of Sephacryl S-300 (superfine), equilibrated with the same buffer. The enzyme activity was assayed as described above and the active fractions eluted in 660–740 ml were pooled and dialysed against 5 mM Tris–HCl buffer, pH 8.0. The dialysate was subjected to FPLC using an anion-exchange Mono Q column (0.5 × 5 cm, Pharmacia). Elution was carried out with a linear gradient from 0 to 0.125 M NaCl in 5 mM Tris–HCl buffer (pH 8.0). The main active fractions were collected and diluted with 5 vols of distilled water and rechromatographed in the same conditions as described above. The effluent was monitored by absorbance at 280 nm. Fractions of 0.5 ml were collected at a flow rate of 1.0 ml min^{–1}. Aliquots (0.2 μ l) of each fraction were assayed by 2 h of incubation with 0.1 nmol of substrate ([Leu]enkephalin–Arg–Arg–Phe–Ala–NH₂) in 100 μ l of 0.1 M Tris–HCl buffer, pH 7.4 (—●—●—●—). The active fraction (marked with bar) was collected and used as the purified enzyme.

Fig. 2 a, SDS-polyacrylamide gel electrophoresis analyses of the purified enzyme in the absence (lane 1) or the presence (lane 2) of the reducing reagent. The purified enzyme obtained from Mono Q chromatography in Fig. 1b was loaded on 2.0-mm slab gel using a 15% polyacrylamide gel and a discontinuous buffer system¹⁸. Proteins were analysed using silver stain method (Bio Rad). Lane 1, 100 ng of the unreduced enzyme. Lane 2, 100 ng of the reduced enzyme, treated with 5% 2-mercaptoethanol at 100 °C for 3 min before application to the gel. The R_F positions of molecular weight standards (bovine serum albumin, MW 68,000; ovalbumin, MW 43,000; soybean trypsin inhibitor, MW 21,500; cytochrome c, MW 14,000) are indicated at the right. **b**, Reverse-phase HPLC analysis of the purified enzyme. One eighth of the purified enzyme obtained from Mono Q chromatography in Fig. 1b was analysed by reverse-phase HPLC on a column (0.4 × 25 cm) of TSK LS-410 ODS-SIL (Toyosoda). Elution was carried out with a linear gradient (24 min) of CH_3CN concentration from 24 to 60% in 0.1% trifluoroacetate solution. Flow rate was 2 ml min⁻¹. The effluent was monitored by absorbance at 210 nm.



producing [Leu]enkephalin-Arg from the substrate was found in fractions 31–38, which were well separated from other proteins (Fig. 1a). The active portion was further purified by gel-filtration on a Sephacryl S-300 column, followed by ion-exchange fast protein liquid chromatography (FPLC) on a Mono Q column and finally a second FPLC on the same column (Fig. 1b), which resolved the enzyme activity as a single protein peak. The purified enzyme was verified to be homogeneous on SDS-polyacrylamide gel electrophoresis both in the absence and in the presence of 5% 2-mercaptoethanol (Fig. 2a), from which we conclude it consists of a single polypeptide chain with a MW of about 43,000, and by analysis on reverse-phase HPLC (Fig. 2b). Although HPLC resulted in a marked decrease of enzyme activity, an appreciable amount of enzyme activity (indicated by bar) was detected only at the position of a single symmetrical protein peak. Starting with 45 g of yeast α -cells, the procedure yielded about 0.1 mg of purified enzyme.

HPLC of reaction products from [Leu]enkephalin-Arg-Arg-Phe-Ala-NH₂ after 2 h of incubation with the purified enzyme at 37 °C resolved three peptide peaks (1–3), which, on the basis of amino acid analyses and comparison of retention time on HPLC with authentic peptides, have been identified as Arg-Phe-Ala-NH₂ (peak 1), Tyr-Gly-Gly-Phe-Leu-Arg (peak 2) and the starting Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Phe-Ala-NH₂ (peak 3). The stoichiometric recovery of reaction products (peak 1, 3.7 nmol; peak 2, 3.6 nmol) suggests that the enzyme exclusively hydrolyses the Arg-Arg bond of the substrate. To characterize the substrate specificity of the enzyme, various peptides were incubated with it and the resultant mixtures analysed on reverse-phase HPLC. We found the enzyme converts [Leu]enkephalin-Lys-Arg-Phe-Ala-NH₂ to [Leu]enkephalin-Lys and Arg-Phe-Ala-NH₂, and [Leu]enkephalin-Arg-Lys-Tyr-Pro-NH₂ to [Leu]enkephalin-Arg and Lys-Tyr-Pro-NH₂, by cleaving the Lys-Arg bond and the Arg-Lys bond respectively (Fig. 3b, c). It catalysed the hydrolysis of X–Y peptide bonds (X, Y: basic residues) of various peptides examined, but peptides containing a single basic residue in the molecule, such as [Leu]enkephalin-Arg-NH₂, [Met]enkephalin-Arg-Phe-NH₂ (Fig. 3d) and [Met]enkephalin-Arg-Gly-Leu, were resistant to the action of the enzyme. Furthermore, typical substrates for trypsin, such as tosyl-L-arginine methyl ester (Tos-Arg-OMe; concentration 1.04 mM)⁸ and benzoyl-L-arginine 4-methyl-coumaryl-7-amide (Bz-Arg-MCA; 50 μ M)⁹, were not affected by the enzyme. When rimorphin, which has both consecutive basic residues (-Arg-Arg-) and a solitary basic residue (-Lys-) in the molecule¹⁰ was examined as a substrate, only the Arg-Arg peptide bond was split by the enzyme (Fig. 3e, Table 1). These results strongly

suggest that the enzyme is an endopeptidase, which specifically attacks the peptide bond between consecutive basic residues. Thus its action is distinct from that of trypsin.

The kinetic constants for [Leu]enkephalin-Arg-Arg-Phe-Ala-NH₂ and [Leu]enkephalin-Lys-Arg-Phe-Ala-NH₂ were determined from Lineweaver-Burk plots with substrate concentrations varying from 0.2 to 1.6 mM. Each substrate was incubated with the enzyme ($[E_0] = 1.09 \times 10^{-8}$ M) for 10 min at 37 °C in 100 μ l of Tris-HCl buffer, pH 7.4. The reaction was stopped by the addition of 100 μ l of 1 M HCl and the amounts of Arg-Phe-Ala-NH₂ released were analysed by amino acid analyses after separating the peptides on reverse-phase HPLC. The apparent K_m and k_{cat} values thus determined were 0.588 mM and 6.82 s⁻¹ for [Leu]enkephalin-Arg-Arg-Phe-Ala-NH₂ and 0.329 mM and 2.52 s⁻¹ for [Leu]enkephalin-Lys-Arg-Phe-Ala-NH₂ respectively.

The effects of pH and various inhibitors on the activity of the enzyme were examined, using [Leu]enkephalin-Arg-Arg-Phe-Ala-NH₂ as a substrate. The optimal pH for enzyme activity was around 7.5, with considerable activity being observed in the wide pH range 5–10. The enzyme was completely inhibited by serine-protease inhibitors such as phenylmethylsulphonyl fluoride and diisopropyl fluorophosphate at concentrations of

Table 1 Substrate specificity of the enzyme, with cleavage sites indicated by arrows

Peptides hydrolysed by the enzyme:

- Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Phe-Ala-NH₂
 Tyr-Gly-Gly-Phe-Leu-Lys-Arg-Phe-Ala-NH₂
 Tyr-Gly-Gly-Phe-Leu-Arg-Lys-Tyr-Pro-NH₂ (β -neoendorphin-NH₂)
 Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile (PH-8P)
 Asp-Tyr-Gln-Lys-Arg-Tyr-Gly-Gly-Phe-Leu
 Tyr-Gly-Gly-Phe-Leu-Arg-Gln-Phe-Lys-Val-Val-Thr (rimorphin)

Peptides not hydrolysed by the enzyme:

- Tyr-Gly-Gly-Phe-Leu-Arg, Tyr-Gly-Gly-Phe-Leu-Lys,
 Tyr-Gly-Gly-Phe-Leu-Arg-NH₂, Tyr-Gly-Gly-Phe-Met-Arg-Phe,
 Tyr-Gly-Gly-Phe-Met-Arg-Phe-NH₂, Tyr-Gly-Gly-Phe-Met-Arg-Gly-Leu,
 Tos-Arg-OMe(TAME), Bz-Arg-MCA

Each peptide (~4 nmol) was incubated with the enzyme in the same conditions as described in Fig. 3 legend. Reaction mixtures were analysed on reverse-phase HPLC and the resultant peptide peaks identified by amino acid analyses. Basic amino acid residues are underlined.

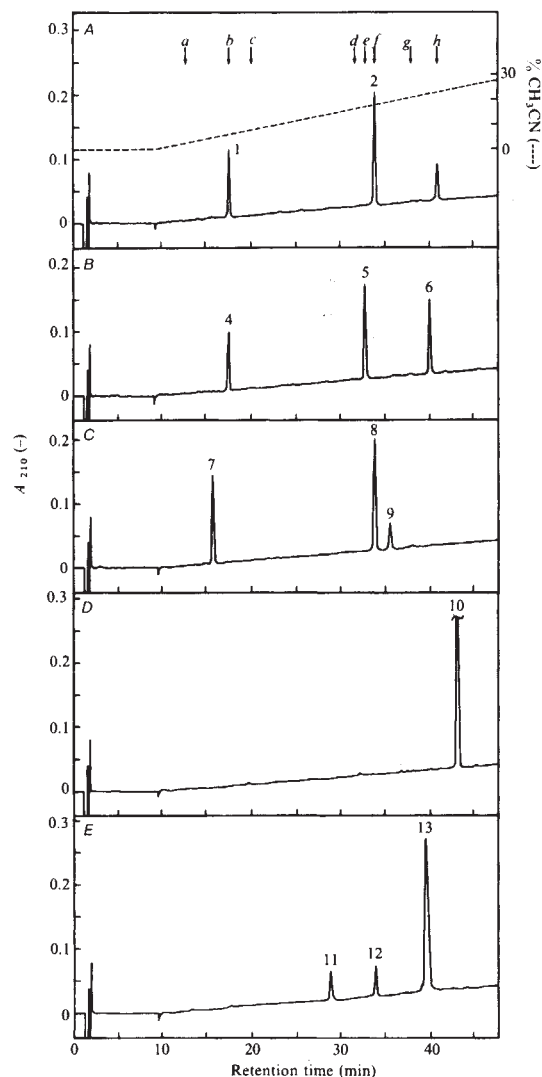


Fig. 3 HPLC patterns of the reaction products from [Leu]enkephalin-Arg-Arg-Phe-Ala-NH₂ (A), [Leu]enkephalin-Lys-Arg-Phe-Ala-NH₂ (B), [Leu]enkephalin-Arg-Lys-Tyr-Pro-NH₂ (C), [Met]enkephalin-Arg-Phe-NH₂ (D) and rimorphin (E), obtained by incubation with the purified enzyme. Approximately 4 nmol of the substrate peptides were incubated at 37 °C for 2 h with 0.1 µg of the purified enzyme in 100 µl of Tris-HCl buffer, pH 7.4. After the reaction was stopped by the addition of 20 µl of 1 M HCl, the solution was directly applied to a column of TSK LS-410 ODS-SIL (0.4 × 25 cm). Elution was carried out with a linear gradient (80 min) of CH₃CN concentration from 0 to 60% in 0.1% trifluoroacetate solution. Flow rate was 2 ml min⁻¹. The effluent was monitored by absorbance at 210 nm. The respective peptide peaks were collected, hydrolysed with 6 M HCl at 110 °C for 22 h, and analysed on an amino acid analyser (Hitachi 835). Based on amino acid composition and comparison of retention time with authentic peptides, the peptide peaks were identified as follows (yields of the peptides are designated in parentheses). A: 1, Arg-Phe-Ala-NH₂ (3.7 nmol); 2, Tyr-Gly-Gly-Phe-Leu-Arg (3.6 nmol); 3, Tyr-Gly-Gly-Phe-Leu-Arg-Phe-Ala-NH₂ (0.7 nmol). B: 4, Arg-Phe-Ala-NH₂ (3.1 nmol); 5, Tyr-Gly-Gly-Phe-Leu-Lys (2.9 nmol); 6, Tyr-Gly-Gly-Phe-Leu-Lys-Arg-Phe-Ala-NH₂ (1.4 nmol). C: 7, Lys-Tyr-Pro-NH₂ (3.5 nmol); 8, Tyr-Gly-Gly-Phe-Leu-Arg (3.4 nmol); 9, Tyr-Gly-Gly-Phe-Leu-Arg-Lys-Tyr-Pro-NH₂ (0.3 nmol). D: 10, Tyr-Gly-Gly-Phe-Met-Arg-Phe-NH₂ (3.9 nmol). E: 11, Arg-Gln-Phe-Lys-Val-Val-Thr (1.0 nmol); 12, Tyr-Gly-Gly-Phe-Leu-Arg (0.98 nmol); 13, Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Gln-Phe-Lys-Val-Val-Thr (2.7 nmol). The elution positions of authentic peptides are indicated by arrows: a, Phe-Ala-NH₂; b, Arg-Phe-Ala-NH₂; c, Arg-Arg-Phe-Ala-NH₂; d, Tyr-Gly-Gly-Phe-Leu-Lys-Arg; e, Tyr-Gly-Gly-Phe-Leu-Lys; f, Tyr-Gly-Gly-Phe-Leu-Arg; g, Tyr-Gly-Gly-Phe-Leu; h, Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Phe-Ala-NH₂.

2×10^{-4} M. Thiol-protease inhibitors, such as monoiodoacetate, and *p*-chloromercuribenzoic acid and metal chelators, such as EDTA and 1,10-phenanthroline at 10^{-3} M, had no effect on enzyme activity. The enzyme can therefore be classified as a serine protease. However, its resistance to the general trypsin inhibitors tosyl-L-lysine chloromethyl ketone (10^{-3} M) and leupeptin (10^{-4} M) indicate that it is distinct from pancreatic trypsin and other related proteases. The recognition mechanism of paired basic residues exhibited by the enzyme was very different from that exhibited by trypsin.

The enzyme described here appears to be a unique and novel endoprotease, showing a substrate specificity and an inhibitor spectrum different from other putative prohormone-processing enzymes¹¹⁻¹⁶. The strict substrate specificity of the enzyme towards paired basic residues indicates that the enzyme may be physiologically involved in the essential function of pro-hormone processing. Further investigation is necessary to demonstrate whether the cleavage at the peptide bond between the paired basic residues generally occurs as the initial proteolytic event in prohormone-processing in tissues.

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Lithium reversibly inhibits microtubule-based motility in sperm flagella

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The sliding-tubule hypothesis of flagellar movement has strong experimental support¹, but our present knowledge of the mechanism by which this sliding is coordinated and converted into flagellar oscillation and bend propagation is quite limited. Among the few facts known are: (1) calcium has a role in regulating the asymmetry of flagellar waveform^{2,3}, (2) the initiation or activation of motility in spermatozoa from several species involves a cyclic AMP-dependent phosphorylation reaction⁴⁻⁷, and (3) the conversion of tubule sliding to bending does not require the radial spokes or central tubules^{8,9}. Inhibitors are valuable tools for investigating mechanisms involved in cellular function, and we report here that Li⁺ in low concentrations reversibly inhibits the microtubule-based movement of reactivated sea urchin sperm flagella. The evidence indicates that the action of Li⁺ is directed primarily towards one or more regulatory sites through which Ca²⁺ modulates the asymmetry of flagellar waveform, rather than towards dynein ATPase

itself. Lithium also appears to inhibit the sperm adenylate cyclase, but this action does not seem to be relevant to its inhibition of normal motility. Our findings indicate the need for considerable caution when using ATP analogues supplied as the Li^+ salt.

Sperm from the sea urchin, *Tripneustes gratilla*, were demembrated in the presence of Ca^{2+} (refs 2, 3) and reactivated at 22–23 °C (Fig. 1). In these conditions the waveforms of the sperm flagella are nearly symmetrical when they are reactivated with Mg-ATP^{2-} at low ($<10^{-6}$ M) Ca^{2+} concentration, becoming asymmetrical only at higher Ca^{2+} concentrations. In the reactivating solution used here, the Mg-ATP^{2-} concentration is 0.5 mM, free Mg^{2+} is 0.1 mM (ref. 10), and the sperm flagella beat at a frequency of 26.0 ± 1.1 Hz (average of six preparations, 69 sperm). Addition of 6–8 mM Li acetate caused abrupt and complete cessation of movement, with the majority of the flagella assuming a straight or almost straight configuration. Dilution of these immobilized sperm 100-fold into fresh reactivating solution without Li^+ immediately restored movement in all of the sperm, with the flagellar beat frequencies and waveforms being indistinguishable from those in the starting preparation. A few experiments were also performed with sperm prepared by demembration in a solution containing 2 mM EGTA and no added Ca^{2+} (refs 2, 3). When these sperm were reactivated with 0.5 mM Mg-ATP^{2-} , their flagellar waveforms were markedly asymmetrical, even in reactivating solution with a low concentration of Ca^{2+} ($<10^{-8}$ M), and addition of 0.1 mM Ca^{2+} induced quiescence, with the sperm flagella assuming a stationary, sharply bent cane-shape³. Addition of Li^+ to such preparations at low Ca^{2+} concentration inhibits the motility similarly to that of sperm demembrated in the presence of Ca^{2+} , except that instead of being nearly straight, the immobilized flagella are bent into cane-shapes resembling the quiescent form produced by Ca^{2+} (refs 3, 11). Addition of 5 mM LiCl to live sperm swimming in seawater had no noticeable effect on motility parameters, but 55 mM LiCl increased waveform asymmetry significantly and decreased longevity.

The standard reactivating solution for this work contained less Mg^{2+} than in our earlier studies because free Mg^{2+} seems to compete with Li^+ . Thus, while 6 mM Li^+ was sufficient to inhibit the sperm in the presence of 0.1 mM free Mg^{2+} , as much as 20 mM Li^+ was needed at 3 mM free Mg^{2+} . Moreover, addition of either 3 mM Mg^{2+} or 6 mM Ca^{2+} , to sperm inhibited initially by 6 mM Li^+ at 0.1 mM free Mg^{2+} , induced the resumption of motility in $>90\%$ of the sperm.

Li^+ at concentrations lower than are needed to completely stop the reactivated movement cause a decrease in flagellar beat frequency (Fig. 1). As the Li^+ concentration is increased, a substantial increase in the asymmetry of the bending waves is observed, and a progressively greater percentage of the sperm become non-motile. Although it becomes difficult to strob the highly asymmetric sperm flagella above 4 mM Li^+ , it appears that none beat at frequencies lower than ~ 17 Hz, corresponding to 65% of the uninhibited value.

The demembrated sperm can be desensitized to the inhibitory effects of Li^+ by brief digestion with trypsin at a trypsin/sperm protein ratio of $\sim 1:10$. Addition of 0.2 μg trypsin ml^{-1} to the reactivating solution containing sperm immobilized with 6–24 mM Li^+ induced a gradual resumption of flagellar beating until after 2–3 min over 90% of the sperm were swimming vigorously. Their waveforms were symmetric and resembled those in undigested control preparations without Li^+ , but their beat frequency was diminished to an extent that depended on the Li^+ concentration (Fig. 2). The rate at which motility was restored by trypsin is approximately the same as the rate at which trypsin eliminated the Ca^{2+} -induced asymmetry of flagellar waveform in a parallel observation dish containing 6 mM Ca^{2+} and no Li^+ (ref. 12).

Li^+ is known to inhibit many adenylate cyclases^{13,14}, and our preliminary experiments have suggested that the cyclase in these sperm is also blocked by Li^+ . In view of this fact, we investigated the effect of cyclic AMP on Li^+ inhibition and found that addition of 10 μM cyclic AMP to preparations inhibited by

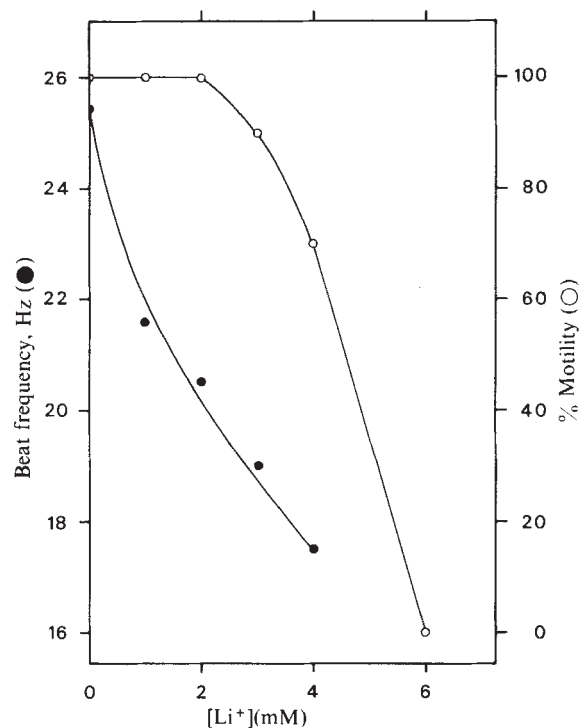


Fig. 1 Decrease in beat frequency (●) and percentage of motile sperm (○) as a function of Li^+ concentration. Sperm were demembrated in a solution containing 0.04% Triton X-100, 4 mM CaCl_2 , 1 mM dithiothreitol (DTT), 0.1 mM EGTA and 10 mM Tris-HCl, pH 8.0 (potentially symmetrical conditions)³ and reactivated at 21.5 °C in a solution containing 0.2 M K acetate, 0.1 mM EGTA, 1 mM DTT, 2% polyethylene glycol (molecular weight 20,000), 0.6 mM MgSO_4 , 1.0 mM ATP, 10 mM Tris-HCl, pH 8.3 (ref. 22), and the indicated concentration of Li acetate.

6 mM Li^+ caused a partial reversal of the inhibition. In most of the 21 preparations observed, 30–70% of the sperm flagella resumed beating with highly asymmetric waveforms, although in a few it was as low as 15% or as high as 90%. The average frequency in these conditions was 18.4 ± 1.4 Hz (4 preparations, 54 sperm), significantly lower than that of sperm flagella desensitized by trypsin digestion, at this Li^+ concentration (Fig. 2). It is unclear what factors influence the degree of reversal obtained with cyclic AMP. Increasing the cyclic AMP concentration to 100 μM had no further effect. However, increasing the Li^+ concentration to 16 mM always resulted in a substantial decrease in the extent of reversal obtained with cyclic AMP, although it did not affect the extent of reversal obtained by dilution; in one such experiment, 25–30% of the sperm resumed swimming in 8 mM Li^+ after addition of 10 μM cyclic AMP, whereas only 0–5% regained motility in 16 mM Li^+ . Addition of 10 μM cyclic GMP instead of cyclic AMP did not reverse the Li^+ inhibition. Also, addition of 10 μM cyclic AMP to control sperm reactivated without Li^+ had no noticeable effect on either the waveforms or frequency in the conditions tested.

To determine whether motility depends on the continuing synthesis of cyclic AMP by adenylate cyclase in normal preparations and whether the inhibition of motility by Li^+ is linked to inhibition of this activity, we replaced the ATP in our reactivating solution with analogues lacking a 3'-hydroxyl group that cannot serve as substrates for adenylate cyclase. Both 3'-deoxy ATP and 2',3'-dideoxy ATP (at 1.0 mM) induce flagellar beating at about 13 Hz and 9 Hz, respectively, with waveforms and longevity very similar to those obtained with ATP. Moreover, this reactivated motility is inhibited by Li^+ , although concentrations of 10–20 mM are required, suggesting that the affinity of these analogues for Mg^{2+} , Li^+ , or for dynein ATPase is different from that of ATP. It seems, therefore, that while Li^+ may block adenylate cyclase activity, this is not the mechanism by which it inhibits flagellar motility.

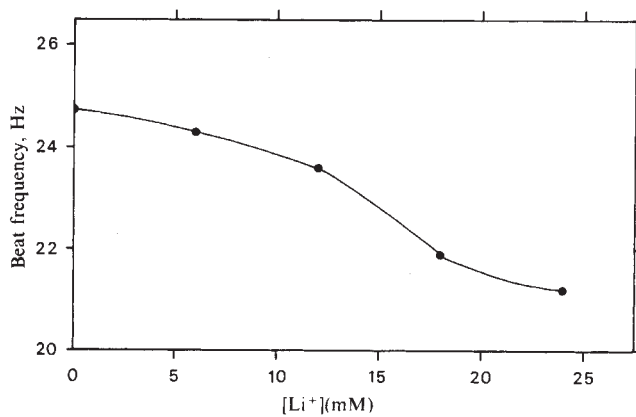


Fig. 2 Decrease in beat frequency as a function of Li^+ concentration of sperm restored to motility by trypsin digestion. Sperm were demembrated and reactivated as described in Fig. 1 legend, then inhibited by addition of Li^+ from 0 to 24 mM, followed by addition of $0.2 \mu\text{g ml}^{-1}$ trypsin. Beat frequencies were read ~2–3 min later, when the flagellar beating most closely resembled that of normal waves. Beyond this period, the movement began to deteriorate until finally the sperm stopped at ~5 min.

Our overall data thus favour a mechanism in which cyclic AMP increases the threshold concentration for Li^+ inhibition of motility, possibly through changing the activation level of the flagellum, while having little effect on the concomitant Li^+ -induced reduction in beat frequency and increase in waveform asymmetry.

Further experiments were designed to test whether Li^+ inhibits dynein arm cross-bridge cycling or ATPase activity. First, we investigated the effect of Li^+ on sperm flagella set into rigor bends in reactivating solution lacking ATP¹⁵, and found that the presence of Li^+ up to 80 mM did not block the straightening of rigor waves that occurred on subsequent addition of 1 mM ATP. Second, we measured the effect of Li^+ on the rate of hydrolysis of Mg-ATP^{2-} by solubilized latent activity dynein 1, and found that it decreased by only 15% in the presence of 4–64 mM Li acetate. On the other hand, the rates of Mg-ATP^{2-} hydrolysis for motile and non-motile (homogenized) preparations of reactivated sperm¹⁶ were decreased from 0.14 ± 0.01 and $0.035 \pm 0.005 \mu\text{mole P}_i$ per min mg protein, respectively, to 0.017 ± 0.003 and $0.013 \pm 0.004 \mu\text{mole P}_i$ per min mg protein in the presence of 10 mM Li^+ in 0.2 M K acetate at pH 8.2. The Li^+ -induced decrease in ATPase activity of motile sperm preparations presumably reflects a loss of movement-coupled ATPase activity resulting directly from immobilization of the sperm flagella.

Our results indicate Li^+ does not inhibit dynein arm cycling *per se*, but rather interacts with a regulatory mechanism—this is shown most clearly by the fact that brief digestion desensitizes the reactivated sperm to inhibition by Li^+ . As low concentrations of Li^+ mimic the effects of Ca^{2+} in inducing asymmetrical beating and quiescent-shaped waveforms, it seems likely that at least one of the regulatory sites concerned is that which normally modulates the Ca^{2+} -dependent asymmetry of flagellar waveform. Besides Ca^{2+} (ref. 3) and Li^+ , the only agents known to inhibit flagellar motility without inhibiting dynein arm cycling activity are CO_2 (ref. 17) and low pH¹⁸. The targets for these agents are unknown, but CO_2 appears to act through an amplitude-regulating mechanism.

The depression of beat frequency (0–13%) that follows desensitization by trypsin in Li^+ concentrations ranging from 0 to 24 mM probably results from a decreased Mg-ATP^{2-} concentration as a consequence of the formation of Li-ATP^{3-} . We calculate that with 6 mM Li^+ added to our reactivating solution, the concentrations of Mg-ATP^{2-} and Li-ATP^{3-} become 0.45 and 0.14 mM, respectively¹⁹, and by direct measurement we have found that Li-ATP^{3-} is not hydrolysed by soluble dynein 1 at a significant rate. However, our present data do not allow us to

say whether Li^+ or Li-ATP^{3-} is the primary form responsible for the increased asymmetry and inhibition of motility in non-trypsin-treated sperm. The apparent competition of free Mg^{2+} or Ca^{2+} with Li^+ could be due to competition for complexing with ATP. However, because many of its effects resemble those of Ca^{2+} , we postulate that Li^+ may be acting as an analogue of Ca^{2+} at a regulatory site that binds Ca^{2+} at elevated Ca^{2+} concentrations, and Mg^{2+} at low Ca^{2+} concentration²⁰. Okuno and Brokaw reported similar difficulty in determining whether Ca^{2+} or Ca-ATP^{3-} interacts with the axoneme to induce either asymmetrical beating, or non-oscillatory beating in the presence of vanadate²¹.

Our work suggests that further study of the effects of Li^+ on cyclic AMP- and Ca^{2+} -dependent regulatory steps may have broad usefulness in unravelling the complex interactions involved in the control of flagellar motility and other cellular functions.

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Rapid mobilization of Ca^{2+} from rat insulinoma microsomes by inositol-1,4,5-trisphosphate

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Several hormones and neurotransmitters raise the cytosolic free Ca^{2+} concentration by stimulating the influx of Ca^{2+} and/or by mobilizing stored Ca^{2+} (refs 1, 2). However, the link between the agonist receptor on the cell surface and the organelle(s) from which Ca^{2+} is mobilized is unknown. One feature of the agonists that increase cytosolic Ca^{2+} is their rapid induction of phosphatidylinositol turnover^{3–8} and polyphosphoinositide hydrolysis^{9–14}; in some tissues this leads, within seconds, to a marked accumulation of the water-soluble products, inositol 1,4-bisphosphate ($\text{Ins}1,4\text{P}_2$) and inositol-1,4,5-trisphosphate ($\text{Ins}1,4,5\text{P}_3$)^{15–18}, suggesting that these might mediate Ca^{2+} mobilization from internal pools^{17,18}. Such an action of $\text{Ins}1,4,5\text{P}_3$ has recently been inferred from studies with permeabilized pancreatic acinar cells¹⁹ and

hepatocytes^{20,21}. Here we show directly that $\text{Ins}1,4,5\text{P}_3$ rapidly releases Ca^{2+} from a microsomal fraction of rat insulinoma but not from mitochondria or secretory granules. Moreover, this response is transient and desensitizes the microsomes to subsequent $\text{Ins}1,4,5\text{P}_3$ additions. These results suggest that $\text{Ins}1,4,5\text{P}_3$ functions as a cellular messenger inducing early mobilization of Ca^{2+} from the endoplasmic reticulum.

The ambient free Ca^{2+} concentration, $[\text{Ca}^{2+}]$, of the incubation medium maintained by the microsomes was determined using a Ca^{2+} -specific minielectrode²². We determined the specific activities of the apparent net Ca^{2+} uptake and of marker enzymes in four fractions obtained after centrifugation of the resuspended microsomes on a sucrose density gradient. Ca^{2+} transport activity correlated strongly with the endoplasmic reticulum enzyme marker but not with the plasma membrane marker (Fig. 1). This demonstrates that the high Ca^{2+} transporting activity of the insulinoma microsomes is localized mainly in the endoplasmic reticulum. As the experiments shown in Fig. 1 required long preparation times and such a large number of insulinomas, all subsequent work was carried out on the original microsomal fraction.

Figure 2a shows that insulinoma microsomes lowered the ambient free Ca^{2+} from $\sim 1.5 \mu\text{M}$ to $\sim 0.1 \mu\text{M}$. At this point a pulse addition of $\text{Ins}1,4,5\text{P}_3$ resulted within 20 s in an increase in medium free Ca^{2+} to $\sim 0.25 \mu\text{M}$, followed by a decrease, representing Ca^{2+} re-uptake. Interestingly, a second addition of $\text{Ins}1,4,5\text{P}_3$ produced only a very small increase in $[\text{Ca}^{2+}]$, indicating that the increase in $[\text{Ca}^{2+}]$ after the first $\text{Ins}1,4,5\text{P}_3$ addition was not due to Ca^{2+} contamination of the compound. When a smaller, first pulse addition of $\text{Ins}1,4,5\text{P}_3$ was made, producing only a modest transient increase in medium $[\text{Ca}^{2+}]$, a second addition of $\text{Ins}1,4,5\text{P}_3$, even at a maximal concentration, elicited only a very small Ca^{2+} response (Fig. 2b). Similar observations were made whether the second addition occurred immediately after the first one or 40 min later. The reason for this phenomenon is unknown; it could conceivably reflect the process of desensitization, which is a feature of Ca^{2+} -mobilizing agonists⁶. Alternatively, during the first stimulation, an $\text{Ins}1,4,5\text{P}_3$ -sensitive vesicle population released Ca^{2+} which was sequestered by a separate population insensitive to the action of $\text{Ins}1,4,5\text{P}_3$. A third possibility is that it was caused by a rapid degradation of $\text{Ins}1,4,5\text{P}_3$ and/or the appearance of degradation products of $\text{Ins}1,4,5\text{P}_3$. Experiments were done to explore this latter hypothesis. When microsomes were incubated in the presence of $9 \mu\text{M}$ $\text{Ins}1,4,5$ [$4,5\text{-}^{32}\text{P}$] P_3 (prepared and analysed as described previously^{16,23}), $85 \pm 7\%$ ($n = 3$) of the label remained after 10 min, indicating that degradation was too slow to account for desensitization. Furthermore, the degradation products of $\text{Ins}1,4,5\text{P}_3$, that is, $\text{Ins}1,4\text{P}_2$, $\text{Ins}1\text{P}$ and *myo*-inositol, all at a concentration of $10 \mu\text{M}$, did not desensitize the microsomes to a subsequent $\text{Ins}1,4,5\text{P}_3$ addition.

The half-maximal effective concentration of $\text{Ins}1,4,5\text{P}_3$ was $3 \mu\text{M}$ and the maximal concentration was $\sim 10 \mu\text{M}$ (Fig. 3). These values are somewhat higher than those reported for permeabilized pancreatic¹⁹ and liver cells^{20,21}. The ability to mobilize Ca^{2+} seems to be specific for the trisphosphate derivative because there was no release of Ca^{2+} on addition of $\text{Ins}1,4\text{P}_2$ (prepared as described previously^{19,23}), $\text{Ins}1\text{P}$ or *myo*-inositol, all tested at $10 \mu\text{M}$ (data not shown).

To test the organelle specificity of the response, insulinoma mitochondria were incubated in conditions similar to the microsome. The mitochondria buffered the ambient $[\text{Ca}^{2+}]$ to $\sim 0.9 \mu\text{M}$ ²². $\text{Ins}1,4,5\text{P}_3$ did not induce Ca^{2+} release nor did it change the extramitochondrial Ca^{2+} steady state. $\text{Ins}1,4,5\text{P}_3$ was not tested at a very low Ca^{2+} concentration ($0.1 \mu\text{M}$) as this causes Ca^{2+} depletion of the mitochondria. Secretory granules were unable either to lower the medium $[\text{Ca}^{2+}]$ or to take up a pulse addition of Ca^{2+} . Furthermore, of the large amount of Ca^{2+} that the secretory granules were found to contain on isolation, none was released by $\text{Ins}1,4,5\text{P}_3$. Thus, neither insulinoma mitochondria nor secretory granules appear to be sensitive to $\text{Ins}1,4,5\text{P}_3$.

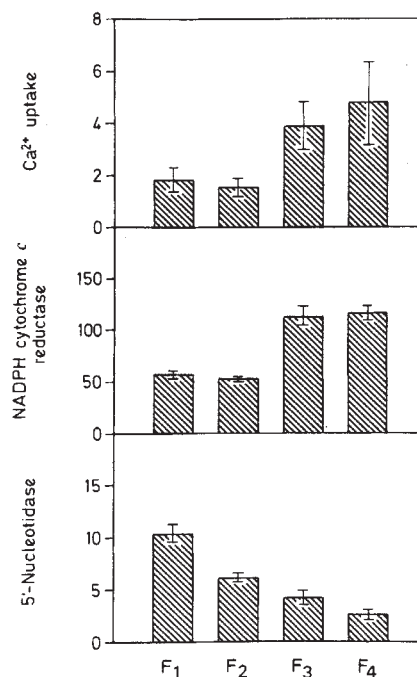
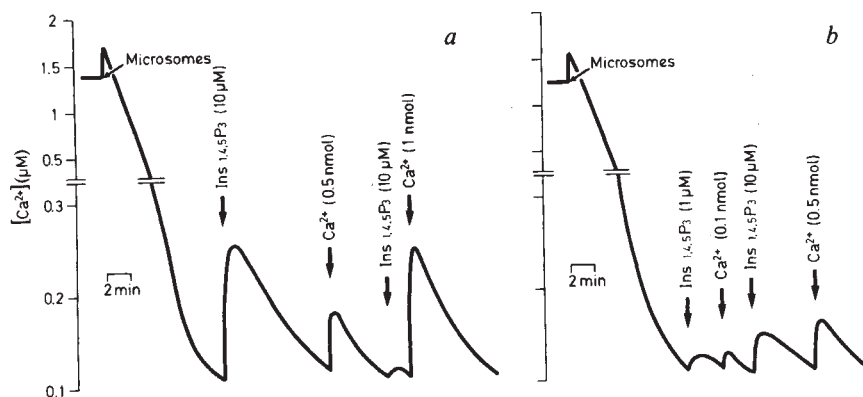


Fig. 1 Specific activities of MgATP -promoted Ca^{2+} uptake and of marker enzymes in four fractions obtained after centrifugation of the resuspended microsomal fraction on a discontinuous sucrose density gradient. Ca^{2+} uptake (nmol of Ca^{2+} per min mg protein) in the four fractions was measured with the same amount of material ($0.2 \text{ mg protein ml}^{-1}$), as in Fig. 2 legend. The amount (nmol) of Ca^{2+} taken up by the four fractions was calculated from the reduction of the ambient free Ca^{2+} concentration on addition of each separate fraction (see also Fig. 2). For NADPH cytochrome c reductase, units are nmol of cytochrome c reduced per min mg protein; for 5'-nucleotidase, units are μmol adenosine formed per h mg protein. Values are the mean $\pm$ s.e. of four experiments.

Methods: Insulinomas were removed from rats^{27,28}, then dissected and homogenized as described previously²². Microsomes were obtained from the supernatant following three successive centrifugations: $1,500g$ for 5 min, $9,800g$ for 10 min, and $24,000g$ for 20 min. The resulting supernatant was centrifuged at $100,000g$ for 60 min to obtain a microsomal pellet. This fraction was enriched in NADPH cytochrome c reductase by 2.6-fold compared with the homogenate, 3.6-fold in 5'-nucleotidase, 0.01-fold in cytochrome c oxidase and 1.3-fold in immunoreactive insulin, the respective markers of endoplasmic reticulum, plasma membrane, mitochondria and secretory granules plus reticular structures. The microsomal pellet was gently washed (without disturbing the pellet) three times with medium containing 0.25 M sucrose, 5 mM HEPES pH 7.0 (sucrose medium) and resuspended in 0.1 ml of the sucrose medium, kept on ice and used immediately for Ca^{2+} transport experiments. In a separate series of experiments, resuspended microsomes were layered onto the surface of a discontinuous sucrose density gradient (1.5 ml of the densities 1.10, 1.14, 1.16, 1.20) and centrifuged at $150,000g$ for 90 min. Four discrete bands observed at the density interfaces were collected for protein, Ca^{2+} flux and enzyme marker determinations. These fractions were numbered 1–4 in order of increasing density (F_1 – F_4). For the isolation of insulin secretory granules, the postnuclear supernatant was centrifuged at $23,700g$ for 15 min. The resultant pellet was resuspended, layered and centrifuged on a 30% Percoll gradient as described previously²². As the bottom of the gradient (see ref. 22) contained mainly insulin secretory granules, this fraction alone was collected. The final secretory granule pellet was obtained after three successive washings in sucrose medium at $23,700g$ for 15 min. The secretory granule fraction was enriched compared with the homogenate 11.2-fold in insulin, 1.1-fold in cytochrome c oxidase and 0.3-fold in NADPH cytochrome c reductase. Cytochrome c oxidase was assayed according to ref. 29; NADPH cytochrome c reductase was measured as described previously³⁰; and 5'-nucleotidase was measured by the technique of Avruch and Wallach³¹. Immunoreactive insulin was assayed according to a method described previously, using rat insulin as standard³². Proteins were determined by the Coomassie blue method using a commercial kit (Bio-Rad).

Fig. 2 Extramicrosomal ambient free Ca^{2+} concentration maintained by insulinoma microsomes: effect of $\text{Ins}1,4,5\text{P}_3$. Microsomes were incubated at 30°C , pH 7.0 in 200 μl of a buffer containing 110 mM KCl, 2 mM KH_2PO_4 , 25 mM HEPES, 1 mM MgCl_2 , 1 mM MgATP, 3 mM creatine phosphate, 50 $\mu\text{g ml}^{-1}$ creatine kinase, 0.2 μM antimycin and 0.5 mg ml^{-1} of bovine serum albumin, with continuous stirring. The medium $[\text{Ca}^{2+}]$ was continuously recorded with a Ca^{2+} -selective minielectrode made and calibrated as described previously²². Where indicated, microsomes (0.75 mg protein ml^{-1}), $\text{Ins}1,4,5\text{P}_3$ or CaCl_2 (nmol per 200 μl) (in the amounts indicated) were added to the medium. Within 10 min, microsomes lowered the extramicrosomal ambient free Ca^{2+} concentration to $\sim 0.1 \mu\text{M}$; they were not easily saturated with Ca^{2+} as they could rapidly take up several sequential pulse additions (5 nmol per mg protein) of Ca^{2+} . The Ca^{2+} accumulated could be rapidly released by adding the Ca^{2+} ionophore A23187 ($1 \mu\text{g ml}^{-1}$) or by lowering the ATP present in the medium by the addition of glucose (5 mM) plus hexokinase (20 U ml^{-1}), indicating that Ca^{2+} is accumulated into vesicular elements in a MgATP-dependent manner (not shown). If microsomes were preincubated with the Ca^{2+} ionophore A23187 to empty the vesicles of Ca^{2+} , the addition of $\text{Ins}1,4,5\text{P}_3$ produced only a very small increase in medium $[\text{Ca}^{2+}]$. This amount of Ca^{2+} corresponded to a similar increase in $[\text{Ca}^{2+}]$ when $\text{Ins}1,4,5\text{P}_3$ was added to the incubation buffer alone; it averaged $\sim 5\text{--}10\%$ of the effect shown in a and was accounted for in the subsequent calculation. $\text{Ins}1,4,5\text{P}_3$ was obtained by alkaline hydrolysis of ox brain phosphatidylinositol-4,5-bisphosphate, followed by preparative paper chromatography (R.F.I. and M.J.B., in preparation). The figure shows representative experiments which were repeated at least eight times.



Three lines of evidence suggest that $\text{Ins}1,4,5\text{P}_3$ induces Ca^{2+} release from the endoplasmic reticulum and not from plasma membrane vesicles contaminating the microsomal fraction. First, the insulinoma microsomal Ca^{2+} uptake correlated well with the endoplasmic reticulum enzyme marker, but not at all with the plasma membrane marker. Second, the amount of Ca^{2+} released by $\text{Ins}1,4,5\text{P}_3$ ($10 \mu\text{M}$) was 6.1 ± 0.2 nmol per mg protein (mean $\pm$ s.e. of six separate experiments). This is a considerable amount of Ca^{2+} —for comparison, most tissues, including pancreatic islets^{1,2}, have a total calcium content of ~ 20 nmol per mg protein. The Ca^{2+} is, therefore, unlikely to originate quantitatively from contaminating plasma membrane vesicles, in a tissue that is particularly rich in reticular structures such as insulinoma cells. Third, $\text{Ins}1,4,5\text{P}_3$ mobilizes Ca^{2+} in permeabilized pancreatic acinar and liver cells in which the plasma membrane should not function as a Ca^{2+} -storing organelle^{19–21}. However, the possibility that $\text{Ins}1,4,5\text{P}_3$ could induce in intact cells an influx of external Ca^{2+} should not be precluded.

Agonist-induced rises in intracellular free $[\text{Ca}^{2+}]$ have been universally associated with an enhancement of both phosphatidylinositol turnover and polyphosphoinositide hydrolysis^{3–18}. More recent observations of a resultant, rapid, Ca^{2+} -independent generation of water-soluble inositol phosphates, have led to the hypothesis that these compounds could act as intracellular messengers mobilizing cellular Ca^{2+} stores^{17,18}. This hypothesis is substantiated and extended by the present report. We have shown that $\text{Ins}1,4,5\text{P}_3$, at concentrations which are thought to occur in cells, can increase, within 20 s, the extramicrosomal concentration of Ca^{2+} from $0.1 \mu\text{M}$ to $\sim 0.2\text{--}$

$0.3 \mu\text{M}$, corresponding respectively to basal or stimulated conditions in a variety of tissues^{24–26}. The results of the present study strongly suggest a specific role for $\text{Ins}1,4,5\text{P}_3$ as a cellular messenger inducing Ca^{2+} release from the endoplasmic reticulum.

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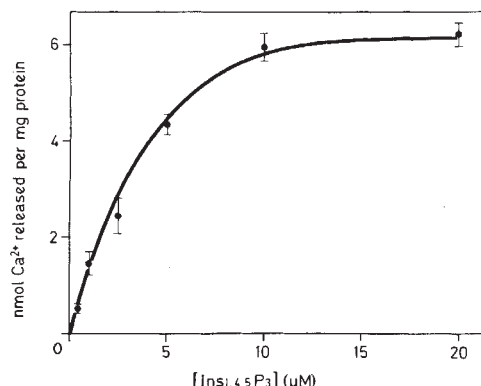


Fig. 3 Dose-response of $\text{Ins}1,4,5\text{P}_3$ -induced Ca^{2+} release. Pulse additions of various known amounts of CaCl_2 (as shown in Fig. 2) were used to calibrate the Ca^{2+} rapidly released by $\text{Ins}1,4,5\text{P}_3$. Values are the mean $\pm$ s.e. of three separate experiments.

Corrigendum

Therapeutic potential of monovalent monoclonal antibodies

S. P. Cobbold & H. Waldmann

Nature **308**, 460–462 (1984)

THE sentence on line 10 of the bold first paragraph should read: 'Starting from the original observation (by Glennie and Stevenson³) that rabbit antisera can... antigens'.

No power of persuasion

Alan Cottrell

Nuclear Power Technology (in three volumes). Edited by W. Marshall. Oxford University Press: 1984. Vol.1 Reactor Technology, pp.503; Vol.2 Fuel Cycle, pp.456; Vol.3 Nuclear Radiation, pp.363. Each volume £35, \$65.

WITHIN this work is an honest tale, plainly told. Therein lies both its strength and weakness. Its strength is that of the scientific tradition for unvarnished presentations of facts, letting them speak for themselves uncoloured by rhetoric, unstrained by advocacy, unheated by passion, unbiased by prejudice. For those raised in the scientific culture this is the natural, indeed the inevitable, mode of expression; the anvil on which every scientific deduction can be mounted and exposed to the hammer of critical analysis. Its weakness is that the scientific culture is a minority one. The great world which fashions public opinion — the world of hyperbole and shock-horror, of the self-inflated politician, bombastic trades union leader, committed journalist, trendy teleperson — resonates to a different sound. The anti-nuclear lobby know this full well. They are at home in this world and play its tunes as captivantly as any Pied Piper.

Thus there is the extraordinary position today — nearly a third of a century after Calder Hall first brought nuclear electricity to Britain, a period in which the nuclear industry has gained a brilliant achievement in supplying cheap power, reliably and safely — that the nuclear industry is on the defensive, trying to justify itself to the general public in the only way it knows, with the quiet voice of scientific reason. For, as Sir Walter Marshall says in his preface to the three volumes, the aim was to prepare a work on nuclear power orientated towards increasing public knowledge and for the public interest. The project was conceived at a time when the United Kingdom Atomic Energy Authority, of which the then Dr Walter Marshall was Chairman, was approaching its twenty-fifth anniversary (in 1979). This event could have justified a little trumpet-blowing, but instead the decision was to review the state of nuclear science and technology in the classical scientific manner.

This is done in 24 chapters, each by different authors who come almost without exception from within the UKAEA. Such a division of the labours is probably the only way to deal comprehensively and authoritatively with such a huge and wide-ranging subject. It has of course the usual advantages and disadvantages. On the merit side each individual topic is given a chapter and author(s) to itself, and so enjoys a reasonably self-contained exposition which can be read separately from the rest of the contents; against this, there is inevitably some repetition of underlying

points that are common to more than one chapter.

The eight chapters in Vol.1 deal with nuclear reactors. After two introductory contributions, which outline the underlying physics and assess the current status of thermal and fast reactors — and go surprisingly far into the mathematics of neutron physics, in a work intended for the public — the various main reactor types are described, namely gas-cooled, light water, fast and heavy water. Two final chapters deal with novel reactor concepts, for example fluid-fuel reactors, and briefly with prospects for fusion power. Volume 2 covers the various aspects of the fuel cycle — occurrence, supply and enrichment of uranium, fuel design and fabrication, reprocessing, waste management, decommissioning, thorium fuel cycles — in similar style. Volume 3, which is perhaps of greatest interest to the general public, deals with various aspects of nuclear radiation — biological, detection, environmental, risk assessment, medical radioisotopes. Radioactive dating is also covered, although surprisingly there is no chapter on the use of gamma radiation in industry or on non-medical applications of radioisotopes.

These three volumes give a splendid tour

d'horizon of the science and technology of nuclear power. Not surprisingly, given their provenance, the expositions centre round British experience and points of view. Nevertheless much of the treatment is sufficiently general as to be of interest to readers in other nations, especially in countries having light-water reactors, for these are dealt with almost as fully as gas-cooled reactors. The treatment of fast reactors is also detailed. Perhaps, if only in fairness to the outstanding Canadian effort, there might have been a little more about the excellent CANDU reactors.

Finally, however, the questions which began this review raise themselves once more. I am sure that many people within the nuclear industry, as well as some scientists and engineers outside it, will read these books with both pleasure and profit. But will the general public and all those publicity-seeking pundits who pronounce so readily and volubly on all matters nuclear? I doubt it. The first group probably does not have the time to digest all the facts so fully gathered together here. The second bask in their unfettered imaginations and have no need of facts. The best response I can make to this work is to thank the authors for a noble effort, which has produced a fine work of science, and to tell them that something entirely different is needed to win the hearts and minds of the great majority in the non-scientific culture. □

Sir Alan Cottrell is Master of Jesus College, Cambridge. Among other books he is author of How Safe is Nuclear Energy? (Heinemann Educational, 1981).

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REASONS

Hormone balance

S.J. Simpson

Endocrinology of Insects. Invertebrate Endocrinology, Vol.1.

Edited by Roger G.H. Downer and Hans Laufer.

Alan R. Liss/Wiley: 1984. Pp.707.

\$199.80, £111.

ONE of the most daunting tasks in biology would be to prepare a volume entitled *Endocrinology of Insects*. So daunting, in fact, that until now no one has attempted to do more than review selected aspects of what is an enormous, complex and rapidly expanding field. Downer and Laufer have approached the problem of producing a detailed, scholarly review of the subject in exactly the right, and probably the only way: they have asked specialists to cover defined ground.

The resulting volume deals with the nature of endocrine glands and neurosecretory cells; the chemistry and biosynthesis of hormones, and their interaction with target cells; intracellular second-messengers; the physiological role of hormones; myotropic

and neurotropic substances; pheromones; the relevance of insect hormones in plants; and the usefulness of hormones in insect control. Inevitably there are omissions, and problems of redundancy and lack of continuity, but the volume as a whole is a success. The differing interpretations and standpoints expressed by the contributors do, as the editors claim, provide depth and balance to the work.

Endocrinology of Insects is not easy to read; not, in most cases, because the writing is unclear, but because the authors have done justice to their topics. This approach has often precluded the drawing of generalizations or syntheses. Even the introductory overviews for such sections as "Regulation of Metamorphosis", "Regulation of [Female] Reproduction" (unfortunately male reproduction is not represented) and "Metabolic Homeostasis" are, by necessity, unable to do more than draw together information in a superficial sense. In most cases, not enough is known to generalize, and what is known is often conflicting — different species use the same hormone for different purposes; different strains of the same species, and even a single strain under different rearing regimes, give conflicting results; it is difficult to distinguish real effects from those stemming from dissection and bioassay techniques; and there is a danger that phenomena are attributed to a particular hormone when in fact the hormone is acting at an earlier stage in a causal sequence involving other endocrine and neural factors. More aspects of insect endocrinology will, perhaps, soon begin to gel, but the cautious approach of many of the contributors is generally justified.

An unusual aspect of the book is the coverage of areas about which virtually nothing is known. Thus the section on metabolic homeostasis includes three detailed chapters on excretion, on lipid metabolism and on carbohydrate metabolism, along with a chapter on nitrogen metabolism in which the word hormone is almost absent. Such a contrast emphasizes those areas which are poorly understood and puts the better known subjects in perspective. The quest for completeness has its drawbacks, however. For example, neurotransmitters, neuro-modulators and pheromones differ from hormones in degree rather than kind and the editors have chosen to represent them but briefly — in the case of pheromones, too briefly to be of much use.

I would have liked more thorough cross-referencing between chapters, but that apart my main worry is that the book is two years out of date. Nonetheless, *Endocrinology of Insects* is a very important work. An understanding of hormonal mechanisms is essential to anyone who studies insect behaviour or physiology, and this book makes a difficult subject accessible to us all. □

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Screening methods of recombination

E.J. Wood

The Techniques in Genetic Engineering Video Library. TGE 1: Nucleic Acids Techniques — An Overview.

By Stephen D.M. Brown.

IRL Press: 1984. Formats available: PAL in VHS and Betamax, £80, \$160. U-matic copies £7.50, \$15 extra. Supplementary charge for NTSC and SECAM standards conversions.

THIS 25-minute videotape is the first of a series of eight* which will deal with the techniques of genetic engineering. Viewed in isolation from the rest of the programmes (which will appear later this year), it gives a foretaste of the high quality of production of the series, but tells us less about what can be expected of the content and level of the subsequent tapes. Nor is it yet clear how easily a student could proceed from instruction of this type to actual work in the laboratory.

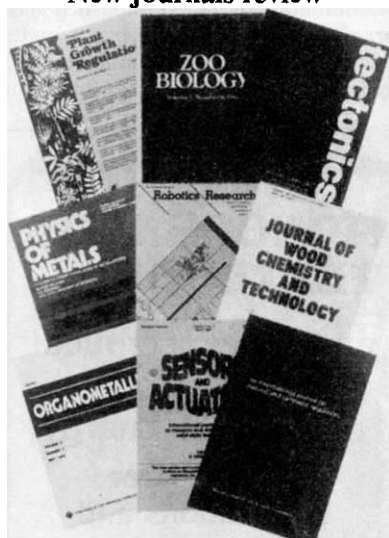
The programme starts with a graphic display of a computer-generated DNA double helix, the introductory section then featuring a series of stills during which the voice-over talks about the impact of genetic engineering and its potential and limitations. A brief interview with Sir Gordon Wolstenholme gives an historical view of developments since Watson and Crick, and stresses that no one really saw any practical uses for the techniques of genetic manipulation until seven or eight years ago.

Following on, the fundamental techniques underlying recombinant DNA technology are explained, namely the use of restriction enzymes, of sticky ends and of ligases. The use of restriction enzymes is well illustrated with graphics, although I wonder if it is really necessary at this level to speak about the different classes of restriction enzyme. Analysis by restriction mapping is demonstrated, and from this a student would readily get a good feel for how slabs of agar are used in electrophoresis, blotting and so on. On the whole this section is evenly paced, well-explained and clearly photographed.

The next section deals with the cloning of DNA into a phage in order "... to look at a single gene". Graphics show the ligation of a section of "human" DNA into phage DNA, reinforcing what had been explained in the previous section. However at this stage the graphics seem to get out of phase with the voice-over: while the latter talks about plaques in a lawn of bacteria, the graphics show thousands of virus particles.

* Titles to follow are *Gene Analysis and Southern Blotting*; *DNA Sequencing with M13*; *Gene Libraries*; *Expression of Cloned Genes*; *Oligonucleotides — Their Use in Gene Cloning*; *Site-Specific Mutagenicity*; and *Microdissection and Microcloning*.

New journals review



On 27 September *Nature* will publish the fourth annual review supplement devoted to science journals.

Criteria for inclusion of a journal in the 1984 issue are that:

(i) the first number appeared, or the journal was retitled, *between June 1982 and May 1983* (the second cut-off date allows at least three issues of a journal to have been published, the minimum number on which a reasonable judgement can be based);

(ii) it is published at least three times a year;

(iii) the main language used is English.

Publishers and learned societies are invited to send *four different issues of each suitable periodical, including the first and most recent numbers* (if from outside the United Kingdom, by air mail) to: The Review Editor, *Nature*, 4 Little Essex Street, London WC2R 3LF, England. Subscription details, if possible for both 1984 and 1985, should be included.

This section of the programme is the most uneven — speeding through a number of techniques (clone selection, blotting, hybridization, DNA sequencing) would surely leave the student wondering what the techniques are for and not really knowing how to carry them out. No doubt such details come in a later programme, but here the producers should have resisted the temptation to pack in so much detail.

The pace then slows down, as cDNA cloning is dealt with briefly but adequately, but speeds up again as we launch into monoclonal antibodies and start injecting things into *Xenopus* oocytes. Well, it is an overview; but again I would have been happier had the desire to mention almost every technique been stifled.

The final section largely uses stills with voice-over to try to answer the question: "Why is industry so interested in all this?" Topics include improving the quality of plant proteins, mineral extraction, "improved" bacteria for energy production from plant waste and for degrading pollutants, as well as interferon and insulin production. Much credit is given to genetic engineering (at the molecular level) for doing, or potentially doing, all of these things; but it is, I think, churlish, if not inaccurate, to fail to mention that for centuries plant and animal breeders have been messing around with the genetic constitutions of species in order to "improve" them.

Also included in the final section is a description of how recombinant DNA technology may, and indeed is, being used in the diagnosis of inherited disease. How this is done is very clearly shown including a description of the analysis of DNA taken from chorionic villi using a DNA probe for prenatal diagnosis of a genetic defect.

Overall the programme gives a good indication for what can be done with recombinant DNA. The photography of the techniques is of a high standard, illustrating the advantage of film or videotape over "cold print" for demonstrating methodology, and most of the graphics are clear and straightforward. The speech, too, is clear and should easily be understood by those whose first language is not English. My main criticism is the unevenness of pace in some parts of the programme, but I would expect this to be less of a problem in subsequent tapes dealing with individual techniques in detail.

A little booklet accompanies the videotape and contains many of the graphics in printed form. These diagrams will be useful for students when revising the details of the techniques. However, while the booklet gives a warning that some of the reagents used are hazardous, neither tape nor booklet says that you should get the permission of a genetic manipulation committee before proceeding. Perhaps this comes later. □

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On ice and climate

Claus U. Hammer

The Climatic Record in Polar Ice Sheets.

Edited by Gordon de Q. Robin.
Cambridge University Press: 1983.
Pp.212. £32.50, \$59.50.

GLACIERS move slowly. So, apparently, does the publication of monographs on polar ice sheets. In *The Climatic Record in Polar Ice Sheets*, an offspring of a workshop held in 1973, the subject is the information to be obtained on past climate from ice sheets, through the past 100,000–150,000 years with a few excursions further back into the Quaternary. The huge polar ice sheets are indeed slowly moving, frozen libraries of past atmospheric conditions, but the information they contain is written in a detailed, complex language. How far have we come in the translation of that language?

The aim of the workshop was to investigate the validity of data obtained from ice sheets, whether in the form of isotopic composition of ice cores or via measured temperature profiles along the corresponding boreholes. Such studies are complicated by the flow history of the ice, and changes in the isotopic composition of the ice can only in part be interpreted as changes in temperature. The book offers a great deal of detailed information on these problems, and a central much-needed contribution (Chapter 5) is devoted to ice flow and heat transport within the ice sheet.

The general reader may have difficulties getting through this account, though glaciologists will feel more at home. The model calculations presented are exemplified by ice core case-studies, but I suspect

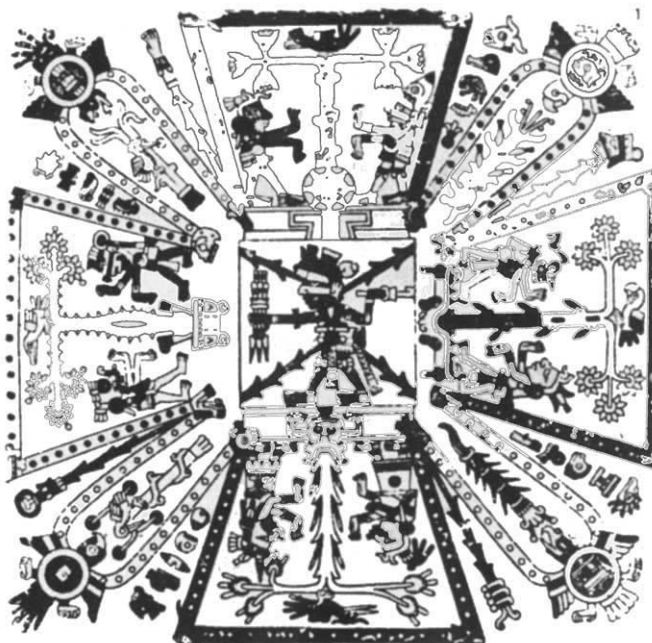
that current models and input parameters are not realistic enough to give safe answers; a lot more glacio-meteorological data are needed before climate may be accurately inferred from ice cores.

In other chapters the reader is given a guided tour of the ice library, but I cannot help feeling a little lost: lacking is an introductory chapter to place the enterprise within the general context of the Quaternary. Attempts to this end have been made in a chapter on ocean cores, but a broader discussion of time scales and variability of ocean core data would have been helpful. Nonetheless, the data presented in the book add much to our understanding of salient problems in this area. For example, the total gas content in air bubbles in the ice can indicate old surface elevations of the ice sheets, and surface studies of snow accumulations, temperatures and so on are clearly of importance.

The book's emphasis on the relationship between isotopes and borehole temperature profiles is more a reflection of its origin at a workshop in 1973 than of new developments. While contributions have been updated by the introduction of more recent results, they do not entirely conceal the fact that ten years have passed since the book was conceived. That being said, some of the most important findings in ice-sheet-climatic research since the 1960s are presented here, among them a number bearing upon still-controversial subjects.

This collection of ice-core data with its discussion of analytical methods is a must for climatic researchers. But to interpret much of the information found in the book such readers may require additional information from the glaciological literature. □

Claus U. Hammer is at the Geophysical Isotope Laboratory, University of Copenhagen.



The cosmological plan of the Aztec universe, laid out horizontally. The illustration shows the nine Lords and two hundred and sixty days of the Sacred Calendar assigned to the four cardinal directions, the centre of the universe, and sacred birds and trees. The illustration, reproduced from the *Codex Fejervary-Mayer I*, is taken from Brian M. Fagan's *The Aztecs*, an illustrated account of early Mexican life and culture. The book is published by W.H. Freeman, price hbk \$27.95, £26.95; pbk \$14.95, £13.95.

Evolution and us

John Beatty

Philosophy, Evolution and Human Nature.

By Florian von Schilcher and Neil Tennant.

Routledge & Kegan Paul: 1984. Pp.283. £16.95, \$29.95.

THIS is a thought-provoking book — the result of a brainstorming collaboration between a philosopher (Tennant) and a behavioural geneticist and evolutionist (von Schilcher). The authors are mainly concerned with the ramifications of adaptationist accounts of human nature. They succeed in demonstrating what one might call (only partly tongue-in-cheek) the "resourcefulness" of the adaptationist perspective, and also do *pretty* well in countering current methodological and epistemological critiques of adaptationist theorizing. But their apparent reasons for making adaptation the centre of attention of a scientific study of human nature include some contentious views about evolutionary biology.

Following a long introduction to the structure and conceptual foundations of evolutionary theory (which is sound, but which could have been improved by incorporating at least *some* of the recent work in philosophy of biology), the authors turn their evolutionary lens upon their own kind — upon human values, cognition and language. As for values, they warn against the "naturalistic fallacy" of those who pretend to derive normative conclusions from purely evolutionary premises, but warn also against the "anti-naturalism" of those who believe that the facts of evolution are entirely irrelevant to ethics. As for cognition, they concentrate upon the "evolutionary exegesis of Kant": to what extent, they ask, are the categories of cognition grounded in neurological structures that are, in turn, the products of evolution by natural selection. They acknowledge, in this regard, some of the problems that result from "spiking" the brain with "an overly rich mixture of cognitive spirits". And, as for language, they are interested in the extent to which the individual development of grammatical skills "recapitulates" the phylogenetic history of grammatical innovations.

Adaptationist accounts of human nature are certainly worth entertaining. But I question a number of Tennant and von Schilcher's underlying reasons for

Paper crust

Princeton University Press have re-issued in paperback *The History of the Earth's Crust*, edited by Robert A. Phinney, the proceedings of a conference held in 1966 which proved to be a landmark in the earth sciences. The book was first published in 1968. Price of paperback is \$11.50, £10.

pursuing such an approach, some of which seem to me to be based on serious misrepresentations of the structure and domain of evolutionary biology. First, they misconstrue evolutionary theory as being underpinned by nothing more than principles of natural selection, and evolutionary biology as being nothing more than the study of adaptations. Then they make unjustifiably bold claims about the domain of evolutionary biology — that is, about how much we can expect to explain in terms of evolution by natural selection.

For instance, among the "components" of the modern synthetic theory of evolution they include "The postulation of natural selection working on random genetic mutations as the one and only mechanism of evolutionary change" (p.97). And yet most versions of the Hardy-Weinberg law (the central principle of the synthetic theory) cite natural selection, mutation, migration, random drift and non-random mating as possible means of evolutionary change.

Even when von Schilcher and Tennant acknowledge alternative mechanisms of evolution, random drift for example, they belittle the search for such phenomena. For instance, they claim that

the pan-selectionist point of view is the only possible working hypothesis. This is simply because someone not searching for a function [i.e. adaptation] will never find one, even if one should exist [p.63].

But surely it would be just as unlikely for someone to find an instance of random drift if they were not looking for it. In other words, there may be a good reason for saying that the pan-selectionist point of

view is the only possible working hypothesis, but von Schilcher and Tennant have not provided it.

Finally, consider the authors' bold *a priori* extension of biological evolutionary theory to cultural evolution:

A priori biological evolution is going to produce culturally adapted individuals when an important part of the environment is cultural; and genotypes placed in such environments will produce phenotypes with important cultural traits [p.113].

And they add:

to discern a causal interaction between genes and culture is not to make a category mistake; indeed, its denial is a logical contradiction once culture is reckoned to the environment, cultural traits are reckoned to the phenotype, and the core conditional of evolutionary theory is understood just as before [p.113].

I believe that the authors have seriously overestimated the power of pure reflection here, and have tried unsuccessfully to turn an empirical, scientific issue into a philosophical one.

In one sense fortunately, and in another sense unfortunately, the authors' stimulating account of the ramifications of an adaptationist approach to human nature does not entirely depend upon their having established the all-importance of evolution by natural selection. It is fortunate in that their discussions, and many of their arguments, are still well worth consideration. It is, however, unfortunate in that the needless mistakes are distracting indeed. □

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Aspects of genius

R.S. Woolhouse

Leibniz. By G. MacDonald Ross.
Oxford University Press: 1984. Pp.121.
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LEIBNIZ (1646–1716) was a multifaceted genius. He was archivist, diplomat, engineer, inventor, librarian, poet and politician. And his vast output of writing covered alchemy, anthropology, dynamics, economics, geology, history, jurisprudence, linguistics, logic, mathematics, numismatics, philosophy and religion.

The introduction to George MacDonald Ross's clear and very readable contribution to the *Past Masters* series prides itself on recognizing this fact. It leads us to expect the novelty of a discussion of all aspects of Leibniz's work and thought. But, after the initial notice taken of them in the valuable account of his life, most are ignored. As usual it is Leibniz's philosophy and parts of his mathematics that really receive the attention.

As even this is a lot to cover in a limited space it need be no surprise if this short

book is not without flaws. Though Leibniz indeed says otherwise, it is not true of the historical Descartes that he squared human behaviour with his law of motion-conservation by making the soul change only the *direction* of motion of brain particles, not the *amount*. And though Leibniz did question how mind could possibly act on body, this was a background metaphysical worry. It was not a particular response to an attempt to make that action consistent with believed empirical laws. His specific objection to the manoeuvre he attributed to Descartes was that though it saved Descartes's (in fact false) law of motion-conservation, it did not save the (true, but unknown to Descartes) law of momentum-conservation.

Yet MacDonald Ross's achievement in introducing and summarizing Leibniz is considerable. Inevitably he compresses and omits. But he does not generally resort to superficialities, or rely on vague and general gestures. As a whole the book is fresh, stimulating and very informative. □

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